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(54) Title: METHOD AND REAGENT FOR THE INHIBITION OF CHECKPOINT KINASE-1 (CHK 1) ENZYME

(57) Abstract: The present invention relates to nucleic acid molecules, including antisense and enzymatic nucleic acid molecules, such as hammerhead ribozymes, DNAzymes, and antisense, which modulate the expression of the Chk-1 gene.

DESCRIPTION

METHOD AND REAGENT FOR THE INHIBITION OF CHECKPOINT KINASE-1 (CHK1) ENZYME

Background Of The Invention

The present invention concerns compounds, compositions, and methods for the study, diagnosis, and treatment of conditions and diseases related to the expression of kinases which phosphorylate Cdc25 S216, such as Chk1 (checkpoint kinase 1) enzyme.

The following is a brief description of the current understanding of Chk1. The discussion is not meant to be complete and is provided only for understanding the invention that follows. The summary is not an admission that any of the work described below is prior art to the claimed invention.

Mammalian cells treated with agents that inhibit DNA replication or cause DNA damage undergo cell cycle arrest due to the presence of multiple checkpoint response mechanisms. Cancer cells frequently lack the p53-induced G1 DNA damage checkpoint response and instead arrest in G2 due to a checkpoint pathway directed towards preventing Cdc2 kinase activation. Inhibition of Cdc2 kinase activity is mediated by Wee1-like kinases, which phosphorylate key residues within the ATP-binding pocket of Cdc2 (accession No. X05360). Maintenance of this arrest also involves repressing Cdc25 function, the phosphatase that removes the Cdc2 inhibitory phosphorylations, by a mechanism involving the binding of 14-3-3 proteins to a phosphorylated serine residue (S216) in Cdc25. Multiple kinases, including Chk1 (accession No. AFO16582), Chk2 (Cds1) (accession No. NM_007194), and C-TAK1 (accession No. AL050393), can phosphorylate Cdc25 S216 (accession No. M34065) *in-vitro*. These kinases may function in the DNA replication and/or DNA damage checkpoint response *in vivo*.

Hoekstra *et al.*, International PCT publication No. WO/9955844, describe, in general terms, a method for promoting differentiation of a differentiation-inhibited cell by introducing into a cell a first polynucleotide encoding an antisense polynucleotide that hybridizes to a second polynucleotide encoding a cell cycle checkpoint protein.

Summary Of The Invention

The invention features novel nucleic acid-based techniques [e.g., enzymatic nucleic acid molecules (ribozymes), antisense nucleic acids, 2-5A antisense chimeras, triplex DNA, antisense nucleic acids containing RNA cleaving chemical groups] and methods for their use to modulate the expression of kinases which phosphorylate Cdc25 S216, such as Chk1 (checkpoint kinase 1) enzyme, Chk2 (Cds1) and C-TAK1.

The description below of the various aspects and embodiments is provided with reference to the exemplary gene Chk1. However, the various aspects and embodiments are also directed to each of the other genes which phosphorylate Cdc25S216. Those additional genes can be analyzed for target sites as described for Chk1. Further, the nucleic acid-based techniques, molecules, and compositions targeted to those genes can be performed as for Chk1. Thus, the inhibition and the effects of such inhibition of the other genes can be performed as described herein.

In a preferred embodiment, the invention features the use of one or more of the nucleic acid-based techniques independently or in combination to inhibit the expression of the genes encoding Chk1. Specifically, the invention features the use of nucleic acid-based techniques to specifically inhibit the expression of Chk1 gene.

In another preferred embodiment, the invention features the use of an enzymatic nucleic acid molecule, preferably in the hammerhead, NCH, G-cleaver, amberzyme, zinzyme and/or DNAzyme motif, to inhibit the expression of Chk1 gene.

By "inhibit" it is meant that the activity of Chk1 or level of RNAs or equivalent RNAs encoding one or more protein subunits of Chk1 is reduced below that observed in the absence of the nucleic acid molecules of the invention. In one embodiment, inhibition with enzymatic nucleic acid molecule preferably is below that level observed in the presence of an enzymatically inactive or attenuated molecule that is able to bind to the same site on the target RNA, but is unable to cleave that RNA. In another embodiment, inhibition with antisense oligonucleotides is preferably below that level observed in the presence of, for example, an oligonucleotide with scrambled sequence or with mismatches. In another embodiment, inhibition of Chk1 genes with the nucleic acid molecule of the instant invention is greater than in the presence of the nucleic acid molecule than in its absence.

By "enzymatic nucleic acid molecule" it is meant a nucleic acid molecule which has complementarity in a substrate-binding region to a specified gene target, and also has an enzymatic activity which is active to specifically cleave target RNA. That is, the enzymatic

nucleic acid molecule is able to intermolecularly cleave RNA and thereby inactivate a target RNA molecule. These complementary regions allow sufficient hybridization of the enzymatic nucleic acid molecule to the target RNA and thus permit cleavage. One hundred percent complementarity is preferred, but complementarity as low as 50-75% may also be useful in this invention (see for example Werner and Uhlenbeck, 1995, *Nucleic Acids Research,* 23, 2092-2096; Hammann *et al.,* 1999, *Antisense and Nucleic Acid Drug Dev.,* 9, 25-31). The nucleic acids may be modified at the base, sugar, and/or phosphate groups. The term enzymatic nucleic acid is used interchangeably with phrases such as ribozymes, catalytic RNA, enzymatic RNA, catalytic DNA, aptazyme or aptamer-binding ribozyme, regulatable ribozyme, catalytic oligonucleotides, nucleozyme, DNAzyme, RNA enzyme, endoribonuclease, endonuclease, minizyme, leadzyme, oligozyme or DNA enzyme. All of these terminologies describe nucleic acid molecules with enzymatic activity. The specific enzymatic nucleic acid molecules described in the instant application are not limiting in the invention and those skilled in the art will recognize that all that is important in an enzymatic nucleic acid molecule of this invention is that it has a specific substrate binding site which is complementary to one or more of the target nucleic acid regions, and that it have nucleotide sequences within or surrounding that substrate binding site which impart a nucleic acid cleaving and/or ligation activity to the molecule (Cech et al., U.S. Patent No. 4,987,071; Cech *et al.*, 1988, 260 *JAMA* 3030).

By "nucleic acid molecule" as used herein is meant a molecule having nucleotides. The nucleic acid can be single, double, or multiple stranded and may comprise modified or unmodified nucleotides or non-nucleotides or various mixtures and combinations thereof.

By "enzymatic portion" or “catalytic domain” is meant that portion/region of the enzymatic nucleic acid molecule essential for cleavage of a nucleic acid substrate (for example, see **Figures 1-5**).

By "substrate binding arm" or "substrate binding domain" is meant that portion/region of a enzymatic nucleic acid which is able to interact, for example via complementarity (*i.e.*, able to base-pair with), with a portion of its substrate. Preferably, such complementarity is 100%, but can be less if desired. For example, as few as 10 bases out of 14 can be base-paired (see for example Werner and Uhlenbeck, 1995, *Nucleic Acids Research,* 23, 2092-2096; Hammann *et al.,* 1999, *Antisense and Nucleic Acid Drug Dev.,* 9, 25-31). Examples of such arms are shown generally in **Figures 1-5**. That is, these arms contain sequences within a enzymatic nucleic acid which are intended to bring enzymatic nucleic acid and target RNA together through complementary base-pairing interactions. The enzymatic nucleic acid of the invention may have binding arms that are contiguous or non-contiguous and may be of varying lengths. The length of the binding arm(s) are preferably greater than or equal to four nucleotides and of sufficient

length to stably interact with the target RNA; preferably 12-100 nucleotides; more preferably 14-24 nucleotides long (see for example Werner and Uhlenbeck, *supra;* Hamman *et al., supra;* Hampel *et al.,* EP0360257; Berzal-Herrance *et al.,* 1993, *EMBO J.,* 12, 2567-73). If two binding arms are chosen, the design is such that the length of the binding arms are symmetrical (*i.e.,* each of the binding arms is of the same length; *e.g.,* five and five nucleotides, or six and six nucleotides, or seven and seven nucleotides long) or asymmetrical (*i.e.,* the binding arms are of different length; *e.g.,* six and three nucleotides; three and six nucleotides long; four and five nucleotides long; four and six nucleotides long; four and seven nucleotides long; and the like).

By "Inozyme" or "NCH" motif is meant, an enzymatic nucleic acid molecule comprising a motif as is generally described as NCH Rz in **Figure 2**. Inozymes possess endonuclease activity to cleave RNA substrates having a cleavage triplet NCH/, where N is a nucleotide, C is cytidine and H is adenosine, uridine or cytidine, and / represents the cleavage site. H is used interchangeably with X. Inozymes can also possess endonuclease activity to cleave RNA substrates having a cleavage triplet NCN/, where N is a nucleotide, C is cytidine, and / represents the cleavage site. "I" in **Figure 2** represents an Inosine nucleotide, preferably a ribo-Inosine or xylo-Inosine nucleoside.

By "G-cleaver" motif is meant, an enzymatic nucleic acid molecule comprising a motif as is generally described as G-cleaver in **Figure 2**. G-cleavers possess endonuclease activity to cleave RNA substrates having a cleavage triplet NYN/, where N is a nucleotide, Y is uridine or cytidine and / represents the cleavage site. G-cleavers may be chemically modified as is generally shown in **Figure 2**.

By "amberzyme" motif is meant, an enzymatic nucleic acid molecule comprising a motif as is generally described in **Figure 3**. Amberzymes possess endonuclease activity to cleave RNA substrates having a cleavage triplet NG/N, where N is a nucleotide, G is guanosine, and / represents the cleavage site. Amberzymes may be chemically modified to increase nuclease stability through substitutions as are generally shown in **Figure 3**. In addition, differing nucleoside and/or non-nucleoside linkers can be used to substitute the 5'-gaaa-3' loops shown in the figure. Amberzymes represent a non-limiting example of an enzymatic nucleic acid molecule that does not require a ribonucleotide (2'-OH) group within its own nucleic acid sequence for activity.

By "zinzyme" motif is meant, an enzymatic nucleic acid molecule comprising a motif as is generally described in **Figure 4**. Zinzymes possess endonuclease activity to cleave RNA substrates having a cleavage triplet including but not limited to YG/Y, where Y is uridine or cytidine, and G is guanosine and / represents the cleavage site. Zinzymes may be chemically

modified to increase nuclease stability through substitutions as are generally shown in **Figure 4**, including substituting 2'-O-methyl guanosine nucleotides for guanosine nucleotides. In addition, differing nucleotide and/or non-nucleotide linkers can be used to substitute the 5'-gaaa-2' loop shown in the figure. Zinzymes represent a non-limiting example of an enzymatic nucleic acid molecule that does not require a ribonucleotide (2'-OH) group within its own nucleic acid sequence for activity.

By 'DNAzyme' is meant, an enzymatic nucleic acid molecule that does not require the presence of a 2'-OH group for its activity. In particular embodiments the enzymatic nucleic acid molecule may have an attached linker(s) or other attached or associated groups, moieties, or chains containing one or more nucleotides with 2'-OH groups. DNAzymes can be synthesized chemically or expressed endogenously *in vivo,* by means of a single stranded DNA vector or equivalent thereof. An example of a DNAzyme is shown in **Figure 5** and is generally reviewed in Usman *et al.*, International PCT Publication No. WO 95/11304; Chartrand *et al.*, 1995, *NAR* 23, 4092; Breaker *et al.*, 1995, *Chem. Bio.* 2, 655; Santoro *et al.*, 1997, *PNAS* 94, 4262; Breaker, 1999, *Nature Biotechnology,* 17, 422-423; and Santoro *et. al.,* 2000, *J. Am. Chem. Soc.,* 122, 2433-39. Additional DNAzyme motifs can be selected for using techniques similar to those described in these references, and hence, are within the scope of the present invention.

By "sufficient length" is meant an oligonucleotide of greater than or equal to 3 nucleotides that is of a length great enough to provide the intended function under the expected condition. For example, for binding arms of enzymatic nucleic acid "sufficient length" means that the binding arm sequence is long enough to provide stable binding to a target site under the expected binding conditions. Preferably, the binding arms are not so long as to prevent useful turnover.

By "stably interact" is meant interaction of the oligonucleotides with target nucleic acid (*e.g.,* by forming hydrogen bonds with complementary nucleotides in the target under physiological conditions) that is sufficient to the intended purpose (e.g., cleavage of target RNA by an enzyme).

By "equivalent" RNA to Chk1 is meant to include those naturally occurring RNA molecules having homology (partial or complete) to Chk1 proteins or encoding for proteins with similar function as Chk1 in various organisms, including human, rodent, primate, rabbit, pig, protozoans, fungi, plants, and other microorganisms and parasites. The equivalent RNA sequence also includes in addition to the coding region, regions such as 5'-untranslated region, 3'-untranslated region, introns, intron-exon junction and the like.

By "homology" is meant the nucleotide sequence of two or more nucleic acid molecules is partially or completely identical.

By "antisense nucleic acid", it is meant a non-enzymatic nucleic acid molecule that binds to target RNA by means of RNA-RNA or RNA-DNA or RNA-PNA (protein nucleic acid; Egholm *et al.,* 1993 *Nature* 365, 566) interactions and alters the activity of the target RNA (for a review, see Stein and Cheng, 1993 *Science* 261, 1004 and Woolf *et al.,* US patent No. 5,849,902). Typically, antisense molecules are complementary to a target sequence along a single contiguous sequence of the antisense molecule. However, in certain embodiments, an antisense molecule may bind to substrate such that the substrate molecule forms a loop, and/or an antisense molecule may bind such that the antisense molecule forms a loop. Thus, the antisense molecule may be complementary to two (or even more) non-contiguous substrate sequences or two (or even more) non-contiguous sequence portions of an antisense molecule may be complementary to a target sequence or both. For a review of current antisense strategies, see Schmajuk *et al.,* 1999, *J. Biol. Chem.,* 274, 21783-21789, Delihas *et al.,* 1997, *Nature,* 15, 751-753, Stein *et al.,* 1997, *Antisense N. A. Drug Dev.,* 7, 151, Crooke, 2000, *Methods Enzymol.,* 313, 3-45; Crooke, 1998, *Biotech. Genet. Eng. Rev.,* 15, 121-157, Crooke, 1997, *Ad. Pharmacol.,* 40, 1-49. In addition, antisense DNA can be used to target RNA by means of DNA-RNA interactions, thereby activating RNase H, which digests the target RNA in the duplex. The antisense oligonucleotides can comprise one or more RNAse H activating region, which is capable of activating RNAse H cleavage of a target RNA. Antisense DNA can be synthesized chemically or expressed via the use of a single stranded DNA expression vector or equivalent thereof.

By "RNase H activating region" is meant a region (generally greater than or equal to 4-25 nucleotides in length, preferably from 5-11 nucleotides in length) of a nucleic acid molecule capable of binding to a target RNA to form a non-covalent complex that is recognized by cellular RNase H enzyme (see for example Arrow *et al.,* US 5,849,902; Arrow *et al.,* US 5,989,912). The RNase H enzyme binds to the nucleic acid molecule-target RNA complex and cleaves the target RNA sequence. The RNase H activating region comprises, for example, phosphodiester, phosphorothioate (preferably at least four of the nucleotides are phosphorothiote substitutions; more specifically, 4-11 of the nucleotides are phosphorothiote substitutions); phosphorodithioate, 5'-thiophosphate, or methylphosphonate backbone chemistry or a combination thereof. In addition to one or more backbone chemistries described above, the RNase H activating region can also comprise a variety of sugar chemistries. For example, the RNase H activating region can comprise deoxyribose, arabino, fluoroarabino or a combination thereof, nucleotide sugar chemistry. Those skilled in the art will recognize that the foregoing are non-limiting examples and that any combination of phosphate, sugar and base chemistry of a nucleic acid that supports the activity of RNase H enzyme is within the scope of the definition of the RNase H activating region and the instant invention.

By "2-5A antisense chimera" is meant an antisense oligonucleotide containing a 5'-phosphorylated 2'-5'-linked adenylate residue. These chimeras bind to target RNA in a sequence-specific manner and activate a cellular 2-5A-dependent ribonuclease which, in turn, cleaves the target RNA (Torrence *et al.,* 1993 *Proc. Natl. Acad. Sci. USA* 90, 1300; Silverman *et al.,* 2000, *Methods Enzymol.,* 313, 522-533; Player and Torrence, 1998, *Pharmacol. Ther.,* 78, 55-113).

By "triplex forming oligonucleotides" is meant an oligonucleotide that can bind to a double-stranded DNA in a sequence-specific manner to form a triple-strand helix. Formation of such triple helix structure has been shown to inhibit transcription of the targeted gene (Duval-Valentin *et al.,* 1992 *Proc. Natl. Acad. Sci. USA* 89, 504; Fox, 2000, *Curr. Med. Chem.,* 7, 17-37; Praseuth *et. al.,* 2000, *Biochim. Biophys. Acta,* 1489, 181-206).

By "gene" it is meant a nucleic acid that encodes an RNA, for example, nucleic acid sequences including but not limited to structural genes encoding a polypeptide.

"Complementarity" refers to the ability of a nucleic acid to form hydrogen bond(s) with another RNA sequence by either traditional Watson-Crick or other non-traditional types. In reference to the nucleic molecules of the present invention, the binding free energy for a nucleic acid molecule with its target or complementary sequence is sufficient to allow the relevant function of the nucleic acid to proceed, e.g., enzymatic nucleic acid cleavage, antisense or triple helix inhibition. Determination of binding free energies for nucleic acid molecules is well known in the art (see, e.g., Turner et al., 1987, *CSH Symp. Quant. Biol.* LII pp.123-133; Frier *et al.,* 1986, *Proc. Nat. Acad. Sci. USA* 83:9373-9377; Turner *et al.,* 1987, *J. Am. Chem. Soc.* 109:3783-3785). A percent complementarity indicates the percentage of contiguous residues in a nucleic acid molecule which can form hydrogen bonds (e.g., Watson-Crick base pairing) with a second nucleic acid sequence (e.g., 5, 6, 7, 8, 9, 10 out of 10 being 50%, 60%, 70%, 80%, 90%, and 100% complementary). "Perfectly complementary" means that all the contiguous residues of a nucleic acid sequence will hydrogen bond with the same number of contiguous residues in a second nucleic acid sequence.

By "RNA" is meant a molecule comprising at least one ribonucleotide residue. By "ribonucleotide" or "2'-OH" is meant a nucleotide with a hydroxyl group at the 2' position of a β-D-ribo-furanose moiety.

By "decoy RNA" is meant a RNA molecule that mimics the natural binding domain for a ligand. The decoy RNA therefore competes with natural binding target for the binding of a specific ligand. For example, it has been shown that over-expression of HIV trans-activation

response (TAR) RNA can act as a "decoy" and efficiently binds HIV tat protein, thereby preventing it from binding to TAR sequences encoded in the HIV RNA (Sullenger et al., 1990, Cell, 63, 601-608). This is but a specific example and those in the art will recognize that other embodiments can be readily generated using techniques generally known in the art.

Several varieties of naturally occurring enzymatic RNAs are known presently. Each can catalyze the hydrolysis of RNA phosphodiester bonds in *trans* (and thus can cleave other RNA molecules) under physiological conditions. **Table I** summarizes some of the characteristics of these ribozymes. In general, enzymatic nucleic acids act by first binding to a target RNA. Such binding occurs through the target binding portion of a enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through complementary base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a target RNA will destroy its ability to direct synthesis of an encoded protein. After an enzymatic nucleic acid has bound and cleaved its RNA target, it is released from that RNA to search for another target and can repeatedly bind and cleave new targets. Thus, a single ribozyme molecule is able to cleave many molecules of target RNA. In addition, the ribozyme is a highly specific inhibitor of gene expression, with the specificity of inhibition depending not only on the base-pairing mechanism of binding to the target RNA, but also on the mechanism of target RNA cleavage. Single mismatches, or base-substitutions, near the site of cleavage can completely eliminate catalytic activity of a ribozyme.

The enzymatic nucleic acid molecule that cleave the specified sites in Chk1-specific RNAs represent a novel therapeutic approach to treat a variety of pathologic indications, including cancer.

In one of the preferred embodiments of the inventions described herein, the enzymatic nucleic acid molecule is formed in a hammerhead or hairpin motif, but may also be formed in the motif of a hepatitis delta virus, group I intron, group II intron or RNase P RNA (in association with an RNA guide sequence), *Neurospora* VS RNA, DNAzymes, NCH cleaving motifs, or G-cleavers. Examples of such hammerhead motifs are described by Dreyfus, *supra,* Rossi *et al.*, 1992, *AIDS Research and Human Retroviruses* 8, 183. Examples of hairpin motifs are described by Hampel *et al.*, EP0360257, Hampel and Tritz, 1989 *Biochemistry* 28, 4929, Feldstein *et al.*, 1989, *Gene* 82, 53, Haseloff and Gerlach, 1989, *Gene*, 82, 43, Hampel *et al.*, 1990 *Nucleic Acids Res.* 18, 299; and Chowrira & McSwiggen, US. Patent No. 5,631,359. The hepatitis delta virus motif is described by Perrotta and Been, 1992 *Biochemistry* 31, 16. The RNase P motif is described by Guerrier-Takada *et al.*, 1983 *Cell* 35, 849; Forster and Altman, 1990, *Science* 249, 783; and Li and Altman, 1996, *Nucleic Acids Res.* 24, 835. The *Neurospora* VS RNA ribozyme

motif is described by Collins (Saville and Collins, 1990 *Cell* 61, 685-696; Saville and Collins, 1991 *Proc. Natl. Acad. Sci. USA* 88, 8826-8830; Collins and Olive, 1993 *Biochemistry* 32, 2795-2799; and Guo and Collins, 1995, *EMBO. J.* 14, 363). Group II introns are described by Griffin *et al.,* 1995, *Chem. Biol.* 2, 761; Michels and Pyle, 1995, *Biochemistry* 34, 2965; and Pyle *et al.,* International PCT Publication No. WO 96/22689. The Group I intron is described by Cech *et al.*, U.S. Patent 4,987,071. DNAzymes are described by Usman *et al.*, International PCT Publication No. WO 95/11304; Chartrand *et al.*, 1995, *NAR* 23, 4092; Breaker *et al.*, 1995, *Chem. Bio.* 2, 655; and Santoro *et al.*, 1997, *PNAS* 94, 4262. NCH cleaving motifs are described in Ludwig & Sproat, International PCT Publication No. WO 98/58058; and G-cleavers are described in Kore *et al.*, 1998, *Nucleic Acids Research* 26, 4116-4120 and Eckstein *et al.*, International PCT Publication No. WO 99/16871. Additional motifs include the Aptazyme (Breaker *et al.,* WO 98/43993), Amberzyme (Class I motif; Figure 3; Beigelman *et al.,* International PCT publication No. WO 99/55857) and Zinzyme (Beigelman *et al.,* International PCT publication No. WO 99/55857), all these references are incorporated by reference herein in their totalities, including drawings and can also be used in the present invention. These specific motifs are not limiting in the invention. and those skilled in the art will recognize that all that is important in an enzymatic nucleic acid molecule of this invention is that it has a specific substrate binding site which is complementary to one or more of the target gene RNA regions, and that it have nucleotide sequences within or surrounding that substrate binding site which impart an RNA cleaving activity to the molecule (Cech *et al.*, U.S. Patent No. 4,987,071).

In preferred embodiments of the present invention, a nucleic acid molecule of the instant invention can be between 13 and 100 nucleotides in length. Exemplary enzymatic nucleic acid molecules of the invention are shown in **Tables III-XIII**. For example, enzymatic nucleic acid molecules of the invention are preferably between 15 and 50 nucleotides in length, more preferably between 25 and 40 nucleotides in length, *e.g.*, 34, 36, or 38 nucleotides in length (for example see Jarvis *et al.,* 1996, *J. Biol. Chem.,* 271, 29107-29112). Exemplary DNAzymes of the invention are preferably between 15 and 40 nucleotides in length, more preferably between 25 and 35 nucleotides in length, *e.g.*, 29, 30, 31, or 32 nucleotides in length (see for example Santoro *et al.,* 1998, *Biochemistry,* 37, 13330-13342; Chartrand *et al.,* 1995, Nucleic Acids Research, 23, 4092-4096). Exemplary antisense molecules of the invention are preferably between 15 and 75 nucleotides in length, more preferably between 20 and 35 nucleotides in length, *e.g.*, 25, 26, 27, or 28 nucleotides in length (see for example Woolf *et al.,* 1992, *PNAS.*, 89, 7305-7309; Milner *et al.,* 1997, *Nature Biotechnology,* 15, 537-541). Exemplary triplex forming oligonucleotide molecules of the invention are preferably between 10 and 40 nucleotides in length, more preferably between 12 and 25 nucleotides in length, *e.g.*, 18, 19, 20, or 21 nucleotides in length (see for example Maher *et al.*, 1990, *Biochemistry,* 29, 8820-8826; Strobel

and Dervan, 1990, *Science,* 249, 73-75). Those skilled in the art will recognize that all that is required is for the nucleic acid molecule are of length and conformation sufficient and suitable for the nucleic acid molecule to catalyze a reaction contemplated herein. The length of the nucleic acid molecules of the instant invention are not limiting within the general limits stated.

Preferably, a nucleic acid molecule that down regulates the replication of Chk1 comprises between 12 and 100 bases complementary to a RNA molecule of Chk1. Even more preferably, a nucleic acid molecule that down regulates the replication of Chk1 comprises between 14 and 24 bases complementary to a RNA molecule of Chk1.

In a preferred embodiment, the invention provides a method for producing a class of nucleic acid–based gene inhibiting agents which exhibit a high degree of specificity for the RNA of a desired target. For example, the enzymatic nucleic acid molecule is preferably targeted to a highly conserved sequence region of target RNAs encoding kinases which phosphorylate Cdc25 S216, such as Chk1 proteins (specifically Chk1 gene) such that specific treatment of a disease or condition can be provided with either one or several nucleic acid molecules of the invention. Such nucleic acid molecules can be delivered exogenously to specific tissue or cellular targets as required. Alternatively, the nucleic acid molecules (*e.g.,* ribozymes and antisense) can be expressed from DNA and/or RNA vectors that are delivered to specific cells.

In a preferred embodiment, the invention features the use of nucleic acid-based inhibitors of the invention to specifically target genes that share homology with the Chk1 gene.

As used in herein "cell" is used in its usual biological sense, and does not refer to an entire multicellular organism, e.g., specifically does not refer to a human. The cell may be present in an organism which may be a human but is preferably a non-human multicellular organism, e.g., birds, plants and mammals such as cows, sheep, apes, monkeys, swine, dogs, and cats. The cell may be prokaryotic (e.g., bacterial cell) or eukaryotic (e.g., mammalian or plant cell).

By "Chk1 proteins" is meant, a protein or a mutant protein derivative thereof, comprising phosphorylation activity, preferably to serine residue (S216), or its equivalent, in Cdc25 phosphatase.

By "highly conserved sequence region" is meant, a nucleotide sequence of one or more regions in a target gene does not vary significantly from one generation to the other or from one biological system to the other.

The nucleic acid-based inhibitors of Chk1 expression are useful for the prevention and/or treatment of diseases and conditions such as cancer, including cancer of the colon, rectum, lung,

breast, prostate and any other diseases or conditions that are related to or will respond to the levels of Chk1 in a cell or tissue, alone or in combination with other therapies. In addition, Chk1 inhibition may be used as a therapeutic target for abrogating the G2 DNA damage checkpoint arrest; a situation that may selectively sensitize p53-deficient tumor cells to radiation or chemotherapy treatment.

By "related" is meant that the reduction of Chk1 expression (specifically Chk1 gene) RNA levels and thus reduction in the level of the respective protein will relieve, to some extent, the symptoms of the disease or condition.

The nucleic acid-based inhibitors of the invention are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells or tissues. The nucleic acid or nucleic acid complexes can be locally administered to relevant tissues *ex vivo*, or *in vivo* through injection, infusion pump or stent, with or without their incorporation in biopolymers. In preferred embodiments, the enzymatic nucleic acid inhibitors comprise sequences, which are complementary to the substrate sequences in **Tables III to VIII**. Examples of such enzymatic nucleic acid molecules also are shown in **Tables III to VIII**. Examples of such enzymatic nucleic acid molecules consist essentially of sequences defined in these Tables.

In yet another embodiment, the invention features antisense nucleic acid molecules and 2-5A chimera including sequences complementary to the substrate sequences shown in **Tables III to IX**. Such nucleic acid molecules can include sequences as shown for the binding arms of the enzymatic nucleic acid molecules in **Tables III to VIII** and sequences shown as GeneBloc™ sequences in **Table IX**. Similarly, triplex molecules can be provided targeted to the corresponding DNA target regions, and containing the DNA equivalent of a target sequence or a sequence complementary to the specified target (substrate) sequence. Typically, antisense molecules will be complementary to a target sequence along a single contiguous sequence of the antisense molecule. However, in certain embodiments, an antisense molecule may bind to substrate such that the substrate molecule forms a loop, and/or an antisense molecule may bind such that the antisense molecule forms a loop. Thus, the antisense molecule may be complementary to two (or even more) non-contiguous substrate sequences or two (or even more) non-contiguous sequence portions of an antisense molecule may be complementary to a target sequence or both.

By "consists essentially of" is meant that the active nucleic acid molecule of the invention, for example an enzymatic nucleic acid molecule, contains an enzymatic center or core equivalent to those in the examples, and binding arms able to bind RNA such that cleavage at the target site occurs. Other sequences may be present which do not interfere with such cleavage. Thus, a core

region may, for example, include one or more loop, stem-loop structure or linker, which does not prevent enzymatic activity. Thus, the underlined regions in the sequences in **Tables III** and **IV** can be such a loop, stem-loop, nucleotide linker, and/or non-nucleotide linker and can be represented generally as sequence "X". For example, a core sequence for a hammerhead enzymatic nucleic acid can comprise a conserved sequence, such as 5'-CUGAUGAG-3' and 5'-CGAA-3' connected by a sequence X, where X is 5'-GCCGUUAGGC-3' (SEQ ID NO 3173) or any other stem II region known in the art or a nucleotide and/or non-nucleotide linker. Similarly, for other nucleic acid molecules of the instant invention, such as Inozyme, G-cleaver, amberzyme, zinzyme, DNAzyme, antisense, 2-5A antisense, triplex forming nucleic acid, and decoy nucleic acids, other sequences or non-nucleotide linkers may be present that do not interfere with the function of the nucleic acid molecule.

Sequence X may be a linker of ≥ 2 nucleotides in length, preferably 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 26, 30, where the nucleotides may preferably be internally base-paired to form a stem of preferably ≥ 2 base pairs. Alternatively or in addition, sequence X may be a non-nucleotide linker. In yet another embodiment, the nucleotide linker X can be a nucleic acid aptamer, such as an ATP aptamer, HIV Rev aptamer (RRE), HIV Tat aptamer (TAR) and others (for a review see Gold *et al.*, 1995, *Annu. Rev. Biochem.*, 64, 763; and Szostak & Ellington, 1993, in *The RNA World*, ed. Gesteland and Atkins, pp. 511, CSH Laboratory Press). A "nucleic acid aptamer" as used herein is meant to indicate a nucleic acid sequence capable of interacting with a ligand. The ligand can be any natural or a synthetic molecule, including but not limited to a resin, metabolites, nucleosides, nucleotides, drugs, toxins, transition state analogs, peptides, lipids, proteins, amino acids, nucleic acid molecules, hormones, carbohydrates, receptors, cells, viruses, bacteria and others.

In yet another embodiment, the non-nucleotide linker X is as defined herein. The term "non-nucleotide" as used herein include either abasic nucleotide, polyether, polyamine, polyamide, peptide, carbohydrate, lipid, or polyhydrocarbon compounds. Specific examples include those described by Seela and Kaiser, *Nucleic Acids Res.* 1990, *18*:6353 and *Nucleic Acids Res.* 1987, *15*:3113; Cload and Schepartz, *J. Am. Chem. Soc.* 1991, *113*:6324; Richardson and Schepartz, *J. Am. Chem. Soc.* 1991, *113*:5109; Ma et al., *Nucleic Acids Res.* 1993, *21*:2585 and *Biochemistry* 1993, *32*:1751; Durand et al., *Nucleic Acids Res.* 1990, *18*:6353; McCurdy et al., *Nucleosides & Nucleotides* 1991, *10*:287; Jschke et al., *Tetrahedron Lett.* 1993, *34*:301; Ono et al., *Biochemistry* 1991, *30*:9914; Arnold *et al.*, International Publication No. WO 89/02439; Usman *et al.*, International Publication No. WO 95/06731; Dudycz *et al.*, International Publication No. WO 95/11910 and Ferentz and Verdine, *J. Am. Chem. Soc.* 1991, *113*:4000, all hereby incorporated by reference herein. A "non-nucleotide" further means any group or

compound which can be incorporated into a nucleic acid chain in the place of one or more nucleotide units, including either sugar and/or phosphate substitutions, and allows the remaining bases to exhibit their enzymatic activity. The group or compound can be abasic in that it does not contain a commonly recognized nucleotide base, such as adenosine, guanine, cytosine, uracil or thymine. Thus, in a preferred embodiment, the invention features an enzymatic nucleic acid molecule having one or more non-nucleotide moieties, and having enzymatic activity to cleave an RNA or DNA molecule.

In another aspect of the invention, ribozymes or antisense molecules that cleave target RNA molecules and inhibit Chk1 (specifically Chk1 gene) activity are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors are preferably DNA plasmids or viral vectors. Ribozyme or antisense expressing viral vectors could be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. Preferably, the recombinant vectors capable of expressing the ribozymes or antisense are delivered as described above, and persist in target cells. Alternatively, viral vectors may be used that provide for transient expression of ribozymes or antisense. Such vectors can be repeatedly administered as necessary. Once expressed, the ribozymes or antisense bind to the target RNA and inhibit its function or expression. Delivery of ribozyme or antisense expressing vectors can be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that would allow for introduction into the desired target cell.

By "vectors" is meant any nucleic acid- and/or viral-based technique used to deliver a desired nucleic acid.

By "patient" is meant an organism, which is a donor or recipient of explanted cells or the cells themselves. "Patient" also refers to an organism to which the nucleic acid molecules of the invention can be administered. Preferably, a patient is a mammal or mammalian cells. More preferably, a patient is a human or human cells.

By "enhanced enzymatic activity" is meant to include activity measured in cells and/or in vivo where the activity is a reflection of both the catalytic activity and the stability of the nucleic acid molecules of the invention. In this invention, the product of these properties can beincreased in vivo compared to an all RNA enzymatic nucleic acid or all DNA enzyme. In some cases, the activity or stability of the nucleic acid molecule can bedecreased (i.e., less than ten-fold), but the overall activity of the nucleic acid molecule is enhanced, in vivo.

The nucleic acid molecules of the instant invention, individually, or in combination or in conjunction with other drugs, can be used to treat diseases or conditions discussed above. For example, to treat a disease or condition associated with the levels of Chk1, the patient may be treated, or other appropriate cells may be treated, as is evident to those skilled in the art, individually or in combination with one or more drugs under conditions suitable for the treatment.

In a further embodiment, the described molecules, such as antisense or ribozymes, can be used in combination with other known treatments to treat conditions or diseases discussed above. For example, the described molecules could be used in combination with one or more known therapeutic agents to treat cancer, including but not limited to cancer of the colon, rectum, lung, breast and prostate.

In another preferred embodiment, the invention features nucleic acid-based inhibitors (*e.g.*, enzymatic nucleic acid molecules (ribozymes), antisense nucleic acids, 2-5A antisense chimeras, triplex DNA, antisense nucleic acids containing RNA cleaving chemical groups) and methods for their use to down regulate or inhibit the expression of genes (*e.g.*, Chk1) capable of progression and/or maintenance of cancer.

In another aspect, the invention provides mammalian cells containing one or more nucleic acid molecules and/or expression vectors of this invention. The one or more nucleic acid molecules may independently be targeted to the same or different sites.

By "comprising" is meant including, but not limited to, whatever follows the word "comprising". Thus, use of the term "comprising" indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present. By "consisting of" is meant including, and limited to, whatever follows the phrase "consisting of". Thus, the phrase "consisting of" indicates that the listed elements are required or mandatory, and that no other elements may be present.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Description Of The Preferred Embodiments

First the drawings will be described briefly.

Drawings

Figure 1 shows the secondary structure model for seven different classes of enzymatic nucleic acid molecules. Arrow indicates the site of cleavage. -------- indicate the target sequence. Lines interspersed with dots are meant to indicate tertiary interactions. - is meant to indicate base-paired interaction. **Group I Intron**: P1-P9.0 represent various stem-loop structures (Cech *et al.,* 1994, *Nature Struc. Bio.,* 1, 273). **RNase P (M1RNA)**: EGS represents external guide sequence (Forster *et al.,* 1990, *Science*, 249, 783; Pace *et al.,* 1990, *J. Biol. Chem.,* 265, 3587). **Group II Intron:** 5'SS means 5' splice site; 3'SS means 3'-splice site; IBS means intron binding site; EBS means exon binding site (Pyle *et al.,* 1994, *Biochemistry*, 33, 2716). **VS RNA:** I-VI are meant to indicate six stem-loop structures; shaded regions are meant to indicate tertiary interaction (Collins, International PCT Publication No. WO 96/19577). **HDV Ribozyme: :** I-IV are meant to indicate four stem-loop structures (Been *et al.,* US Patent No. 5,625,047). **Hammerhead Ribozyme: :** I-III are meant to indicate three stem-loop structures; stems I-III can be of any length and may be symmetrical or asymmetrical (Usman *et al.,* 1996, *Curr. Op. Struct. Bio.,* 1, 527). **Hairpin Ribozyme:** Helix 1, 4 and 5 can be of any length; Helix 2 is between 3 and 8 base-pairs long; Y is a pyrimidine; Helix 2 (H2) is provided with a least 4 base pairs (*i.e.,* n is 1, 2, 3 or 4) and helix 5 can be optionally provided of length 2 or more bases (preferably 3 - 20 bases, *i.e.,* m is from 1 - 20 or more). Helix 2 and helix 5 may be covalently linked by one or more bases (*i.e.,* r is ≥ 1 base). Helix 1, 4 or 5 may also be extended by 2 or more base pairs (*e.g.,* 4 - 20 base pairs) to stabilize the ribozyme structure, and preferably is a protein binding site. In each instance, each N and N' independently is any normal or modified base and each dash represents a potential base-pairing interaction. These nucleotides may be modified at the sugar, base or phosphate. Complete base-pairing is not required in the helices, but is preferred. Helix 1 and 4 can be of any size (*i.e.,* o and p is each independently from 0 to any number, *e.g.,* 20) as long as some base-pairing is maintained. Essential bases are shown as specific bases in the structure, but those in the art will recognize that one or more may be modified chemically (abasic, base, sugar and/or phosphate modifications) or replaced with another base without significant effect. Helix 4 can be formed from two separate molecules, *i.e.,* without a connecting loop. The connecting loop when present may be a ribonucleotide with or without modifications to its base, sugar or phosphate. "q" ≥ is 2 bases. The connecting loop can also be replaced with a non-nucleotide linker molecule. H refers to bases A, U, or C. Y refers to pyrimidine bases. "_____" refers to a covalent bond. (Burke *et al.,* 1996, *Nucleic Acids & Mol. Biol.,* 10, 129; Chowrira *et al.,* US Patent No. 5,631,359).

Figure 2 shows examples of chemically stabilized ribozyme motifs. **HH Rz**, represents hammerhead ribozyme motif (Usman *et al.,* 1996, *Curr. Op. Struct. Bio.,* 1, 527); **NCH Rz** represents the NCH ribozyme motif (Ludwig & Sproat, International PCT Publication No. WO

98/58058); **G-Cleaver**, represents G-cleaver ribozyme motif (Kore *et al.*, 1998, *Nucleic Acids Research* 26, 4116-4120). **N** or **n**, represent independently a nucleotide which may be same or different and have complementarity to each other; **rI**, represents ribo-Inosine nucleotide; arrow indicates the site of cleavage within the target. Position 4 of the HH Rz and the NCH Rz is shown as having 2'-*C*-allyl modification, but those skilled in the art will recognize that this position can be modified with other modifications well known in the art, so long as such modifications do not significantly inhibit the activity of the ribozyme.

Figure 3 shows an example of the Amberzyme ribozyme motif that is chemically stabilized (see, for example, Beigelman *et al.*, International PCT publication No. WO 99/55857, incorporated by reference herein; also referred to as Class I Motif). The Amberzyme motif is a class of enzymatic nucleic molecules that do not require the presence of a ribonucleotide (2'-OH) group for its activity.

Figure 4 shows an example of the Zinzyme A ribozyme motif that is chemically stabilized (Beigelman *et al.*, International PCT publication No. WO 99/55857, incorporated by reference herein; also referred to as Class A or Class II Motif). The Zinzyme motif is a class of enzymatic nucleic molecules that do not require the presence of a ribonucleotide (2'-OH) group for its activity.

Figure 5 shows an example of a DNAzyme motif described by Santoro *et al.*, 1997, *PNAS*, 94, 4262.

Figure 6 shows a bar graph of a nucleic acid inhibitor (50 to 200 nM GeneBloc™ screen against Chk1 RNA in HeLa cells using 1.25 μg/ml GSV lipid with 24 hour sustained delivery in a 96-well format. Relative amounts of target RNA were measured normalized to actin using real-time PCR monitoring of amplification compared to mismatch nucleic acid and untreated controls. The sequences of GeneBloc™ reagents used in this experiment are shown in **Table IX**.

Figure 7 shows a bar graph of a lipid optimization study utilizing lead nucleic acid inhibitors (GeneBlocs™) targeting Chk1 RNA in HeLa cells; 96-well plate format, 5000 cells/well, GSV lipid. Six different lipid concentrations are shown in conjunction with two different concentrations of the nucleic acid inhibitors.

Figure 8 shows a bar graph displaying a time-course inhibition study of a lead nucleic acid inhibitor (GeneBloc™) targeting Chk1 RNA compared to a scrambled nucleic acid control, both at 5 and 100 nM concentrations; 96-well plate format, 5000 cells/well, 1.0 μg/ml GSV lipid.

Figure 9 shows a bar graph representing inhibition of Chk1 RNA via primary lead (GeneBloc™) inhibition as described in **Figure 6**, however utilizing a 6-well plate format with a cell density of 150,000 cells per well.

Figure 10 shows a bar graph representing inhibition of Chk1 RNA via primary lead (GeneBloc™) inhibition in conjunction with +/- etoposide and nocodazole treatment; 50 nM GeneBloc™, 1.25 μg/ml GSV lipid, HeLa cells, 6-well plate format, 100,000 cells/well.

Figure 11 shows a bar graph of a lipid optimization study utilizing a lead nucleic acid inhibitor (GeneBloc™) targeting Chk1 RNA in DLD-1 cells; 96-well plate format, 15,000 cells/well, GSV lipid. Four different lipid concentrations are shown in conjunction with two different concentrations of the nucleic acid inhibitor.

Figure 12 shows a bar graph of a lipid optimization study utilizing a lead nucleic acid inhibitor (GeneBloc™) targeting Chk1 RNA in MCF-7 cells; 96-well plate format, 10,000 cells/well, GSV lipid. Four different lipid concentrations are shown in conjunction with two different concentrations of the nucleic acid inhibitor.

Figure 13 shows a dose curve of primary and secondary nucleic acid inhibitor (GeneBloc™) leads targeting Chk1 RNA in HeLa cells using 1.25 μg/ml GSV lipid, 24 hr time-point, 96-well plate format with a density of 5000 cells/well.

Mechanism of action of Nucleic Acid Molecules of the Invention

Antisense: Antisense molecules can be modified or unmodified RNA, DNA, or mixed polymer oligonucleotides which primarily function by specifically binding to matching sequences resulting in inhibition of peptide synthesis (Wu-Pong, Nov 1994, *BioPharm*, 20-33). The antisense oligonucleotide binds to target RNA by Watson Crick base-pairing and blocks gene expression by preventing ribosomal translation of the bound sequences either by steric blocking or by activating RNase H enzyme. Antisense molecules can also alter protein synthesis by interfering with RNA processing or transport from the nucleus into the cytoplasm (Mukhopadhyay & Roth, 1996, *Crit. Rev. in Oncogenesis* 7, 151-190).

In addition, binding of single stranded DNA to RNA may result in nuclease degradation of the heteroduplex (Wu-Pong, *supra*; Crooke, *supra*). To date, the only backbone modified DNA chemistry which will act as substrates for RNase H are phosphorothioates, phosphorodithioates, and borontrifluoridates. Recently it has been reported that 2'-arabino and 2'-fluoro arabino-containing oligos can also activate RNase H activity.

A number of antisense molecules have been described that utilize novel configurations of chemically modified nucleotides, secondary structure, and/or RNase H substrate domains (Woolf *et al.*, International PCT Publication No. WO 98/13526; Thompson *et al.*, International PCT Publication No. WO 99/54459; Hartmann *et al.*, USSN 60/101,174 which was filed on September 21, 1998) all of these are incorporated by reference herein in their entirety.

In addition, antisense deoxyoligoribonucleotides can be used to target RNA by means of DNA-RNA interactions, thereby activating RNase H, which digests the target RNA in the duplex. Antisense DNA can be expressed via the use of a single stranded DNA intracellular expression vector or equivalents and variations thereof.

Triplex Forming Oligonucleotides (TFO): Single stranded DNA can be designed to bind to genomic DNA in a sequence specific manner. TFOs are comprised of pyrimidine-rich oligonucleotides which bind DNA helices through Hoogsteen Base-pairing (Wu-Pong, *supra*). The resulting triple helix composed of the DNA sense, DNA antisense, and TFO disrupts RNA synthesis by RNA polymerase. The TFO mechanism can result in gene expression or cell death since binding may be irreversible (Mukhopadhyay & Roth, *supra*).

2-5A Antisense Chimera: The 2-5A system is an interferon mediated mechanism for RNA degradation found in higher vertebrates (Mitra *et al.*, 1996, *Proc Nat Acad Sci USA* 93, 6780-6785). Two types of enzymes, 2-5A synthetase and RNase L, are required for RNA cleavage. The 2-5A synthetases require double stranded RNA to form 2'-5' oligoadenylates (2-5A). 2-5A then acts as an allosteric effector for utilizing RNase L which has the ability to cleave single stranded RNA. The ability to form 2-5A structures with double stranded RNA makes this system particularly useful for inhibition of viral replication.

(2'-5') oligoadenylate structures can be covalently linked to antisense molecules to form chimeric oligonucleotides capable of RNA cleavage (Torrence, *supra*). These molecules putatively bind and activate a 2-5A dependent RNase, the oligonucleotide/enzyme complex then binds to a target RNA molecule which can then be cleaved by the RNase enzyme.

Enzymatic Nucleic Acid: Seven basic varieties of naturally occurring enzymatic RNAs are presently known. In addition, several *in vitro* selection (evolution) strategies (Orgel, 1979, *Proc. R. Soc. London,* B 205, 435) have been used to evolve new nucleic acid catalysts capable of catalyzing cleavage and ligation of phosphodiester linkages (Joyce, 1989, *Gene,* 82, 83-87; Beaudry *et al.,* 1992, *Science* 257, 635-641; Joyce, 1992, *Scientific American* 267, 90-97; Breaker *et al.,* 1994, *TIBTECH* 12, 268; Bartel *et al.,*1993, *Science* 261:1411-1418; Szostak, 1993, *TIBS* 17, 89-93; Kumar *et al.,* 1995, *FASEB J.,* 9, 1183; Breaker, 1996, *Curr. Op. Biotech.,* 7, 442; Santoro *et al.,* 1997, *Proc. Natl. Acad. Sci.,* 94, 4262; Tang *et al.,* 1997, *RNA* 3, 914;

Nakamaye & Eckstein, 1994, *supra*; Long & Uhlenbeck, 1994, *supra*; Ishizaka *et al.*, 1995, *supra*; Vaish *et al.*, 1997, *Biochemistry* 36, 6495; all of these are incorporated by reference herein). Each can catalyze a series of reactions including the hydrolysis of phosphodiester bonds in *trans* (and thus can cleave other RNA molecules) under physiological conditions.

Nucleic acid molecules of this invention will block to some extent Chk1 protein expression and can be used to treat disease or diagnose disease associated with the levels of Chk1.

The enzymatic nature of a ribozyme has significant advantages, such as the concentration of ribozyme necessary to affect a therapeutic treatment is lower. This advantage reflects the ability of the ribozyme to act enzymatically. Thus, a single ribozyme molecule is able to cleave many molecules of target RNA. In addition, the ribozyme is a highly specific inhibitor, with the specificity of inhibition depending not only on the base-pairing mechanism of binding to the target RNA, but also on the mechanism of target RNA cleavage. Single mismatches, or base-substitutions, near the site of cleavage can be chosen to completely eliminate catalytic activity of a ribozyme.

Nucleic acid molecules having an endonuclease enzymatic activity are able to repeatedly cleave other separate RNA molecules in a nucleotide base sequence-specific manner. Such enzymatic nucleic acid molecules can be targeted to virtually any RNA transcript, and achieve efficient cleavage *in vitro* (Zaug *et al.*, 324, *Nature* 429 1986 ; Uhlenbeck, 1987 *Nature* 328, 596; Kim *et al.*, 84 *Proc. Natl. Acad. Sci. USA* 8788, 1987; Dreyfus, 1988, *Einstein Quart. J. Bio. Med.*, 6, 92; Haseloff and Gerlach, 334 *Nature* 585, 1988; Cech, 260 *JAMA* 3030, 1988; and Jefferies *et al.*, 17 *Nucleic Acids Research* 1371, 1989; Santoro *et al.*, 1997 *supra*).

Because of their sequence specificity, *trans*-cleaving ribozymes can be used as therapeutic agents for human disease (Usman & McSwiggen, 1995 *Ann. Rep. Med. Chem.* 30, 285-294; Christoffersen and Marr, 1995 *J. Med. Chem.* 38, 2023-2037). Ribozymes can be designed to cleave specific RNA targets within the background of cellular RNA. Such a cleavage event renders the RNA non-functional and abrogates protein expression from that RNA. In this manner, synthesis of a protein associated with a disease state can be selectively inhibited (Warashina *et al.*, 1999, *Chemistry and Biology*, 6, 237-250).

The nucleic acid molecules of the instant invention are also referred to as GeneBloc™ reagents, which are essentially nucleic acid molecules (e.g.; ribozymes, antisense) capable of down-regulating gene expression.

GeneBlocs are modified oligonucleotides including ribozymes and modified antisense oligonucleotides that bind to and target specific mRNA molecules. Because GeneBlocs can be

designed to target any specific mRNA, their potential applications are quite broad. Traditional antisense approaches have often relied heavily on the use of phosphorothioate modifications to enhance stability in biological samples, leading to a myriad of specificity problems stemming from non-specific protein binding and general cytotoxicity (Stein, 1995, *Nature Medicine*, 1, 1119). In contrast, GeneBlocs contain a number of modifications that confer nuclease resistance while making minimal use of phosphorothioate linkages, which reduces toxicity, increases binding affinity and minimizes non-specific effects compared with traditional antisense oligonucleotides. Similar reagents have recently been utilized successfully in various cell culture systems (Vassar, *et al.*, 1999, *Science*, 286, 735) and *in vivo* (Jarvis et al., manuscript in preparation). In addition, novel cationic lipids can be utilized to enhance cellular uptake in the presence of serum. Since ribozymes and antisense oligonucleotides regulate gene expression at the RNA level, the ability to maintain a steady-state dose of GeneBloc over several days was important for target protein and phenotypic analysis. The advances in resistance to nuclease degradation and prolonged activity *in vitro* have supported the use of GeneBlocs in target validation applications.

Target sites

Targets for useful ribozymes and antisense nucleic acids can be determined as disclosed in Draper *et al.*, WO 93/23569; Sullivan *et al.*, WO 93/23057; Thompson *et al.*, WO 94/02595; Draper *et al.*, WO 95/04818; McSwiggen *et al.*, US Patent No. 5,525,468. All of these publications are hereby incorporated by reference herein in their totality. Other examples include the following PCT applications, which concern inactivation of expression of disease-related genes: WO 95/23225, WO 95/13380, WO 94/02595, all of which are incorporated by reference herein. Rather than repeat the guidance provided in those documents here, specific examples of such methods are provided herein, not limiting to those in the art. Ribozymes and antisense to such targets are designed as described in those applications and synthesized to be tested *in vitro* and *in vivo*, as also described. The sequences of human Chk1 RNAs were screened for optimal enzymatic nucleic acid and antisense target sites using a computer-folding algorithm. Antisense, hammerhead, DNAzyme, NCH, amberzyme, zinzyme, or G-Cleaver ribozyme binding/cleavage sites were identified. These sites are shown in **Tables III to VIII** (all sequences are 5' to 3' in the tables; underlined regions can be any sequence or linker X, the actual sequence is not relevant here). The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of enzymatic nucleic acid molecule. While human sequences can be screened and enzymatic nucleic acid molecule and/or antisense thereafter designed, as discussed in Stinchcomb *et al.*, WO 95/23225, mouse targeted ribozymes may be useful to test efficacy of action of the enzymatic nucleic acid molecule and/or antisense prior to testing in humans.

Antisense, hammerhead, DNAzyme, NCH, amberzyme, zinzyme or G-Cleaver ribozyme binding/cleavage sites were identified. The nucleic acid molecules are individually analyzed by computer folding (Jaeger *et al.,* 1989 *Proc. Natl. Acad. Sci. USA*, 86, 7706) to assess whether the sequences fold into the appropriate secondary structure. Those nucleic acid molecules with unfavorable intramolecular interactions such as between the binding arms and the catalytic core are eliminated from consideration. Varying binding arm lengths can be chosen to optimize activity.

Antisense, hammerhead, DNAzyme, NCH, amberzyme, zinzyme or G-Cleaver ribozyme binding/cleavage sites were identified and were designed to anneal to various sites in the RNA target. The binding arms are complementary to the target site sequences described above. The nucleic acid molecules were chemically synthesized. The method of synthesis used follows the procedure for normal DNA/RNA synthesis as described below and in Usman *et al.,* 1987 *J. Am. Chem. Soc.*, 109, 7845; Scaringe *et al.,* 1990 *Nucleic Acids Res.*, 18, 5433; Wincott *et al.,* 1995 *Nucleic Acids Res.* 23, 2677-2684; and Caruthers *et al.*, 1992, *Methods in Enzymology* 211,3-19.

Synthesis of Nucleic acid Molecules

Synthesis of nucleic acids greater than 100 nucleotides in length is difficult using automated methods, and the therapeutic cost of such molecules is prohibitive. In this invention, small nucleic acid motifs ("small refers to nucleic acid motifs no more than 100 nucleotides in length, preferably no more than 80 nucleotides in length, and most preferably no more than 50 nucleotides in length; *e.g.*, antisense oligonucleotides, hammerhead or the NCH ribozymes) are preferably used for exogenous delivery. The simple structure of these molecules increases the ability of the nucleic acid to invade targeted regions of RNA structure. Exemplary molecules of the instant invention are chemically synthesized, and others can similarly be synthesized.

Oligonucleotides (e.g.; antisense GeneBlocs™) are synthesized using protocols known in the art as described in Caruthers *et al.*, 1992, *Methods in Enzymology* 211, 3-19, Thompson *et al.,* International PCT Publication No. WO 99/54459, Wincott *et al.,* 1995, *Nucleic Acids Res.* 23, 2677-2684, Wincott *et al.,* 1997, *Methods Mol. Bio.*, 74, 59, Brennan *et al.,* 1998, *Biotechnol Bioeng.,* 61, 33-45, and Brennan, US patent No. 6,001,311. All of these references are incorporated herein by reference. The synthesis of oligonucleotides makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. In a non-limiting example, small scale syntheses are conducted on a 394 Applied Biosystems, Inc. synthesizer using a 0.2 μmol scale protocol with a 2.5 min coupling step for 2'-O-methylated nucleotides and a 45 sec coupling step for 2'-deoxy nucleotides. **Table II** outlines the amounts and the contact times of the reagents used in the

synthesis cycle. Alternatively, syntheses at the 0.2 μmol scale can be performed on a 96-well plate synthesizer, such as the instrument produced by Protogene (Palo Alto, CA) with minimal modification to the cycle. A 33-fold excess (60 μL of 0.11 M = 6.6 μmol) of 2'-O-methyl phosphoramidite and a 105-fold excess of S-ethyl tetrazole (60 μL of 0.25 M = 15 μmol) can be used in each coupling cycle of 2'-O-methyl residues relative to polymer-bound 5'-hydroxyl. A 22-fold excess (40 μL of 0.11 M = 4.4 μmol) of deoxy phosphoramidite and a 70-fold excess of S-ethyl tetrazole (40 μL of 0.25 M = 10 μmol) can be used in each coupling cycle of deoxy residues relative to polymer-bound 5'-hydroxyl. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by colorimetric quantitation of the trityl fractions, are typically 97.5-99%. Other oligonucleotide synthesis reagents for the 394 Applied Biosystems, Inc. synthesizer include; detritylation solution is 3% TCA in methylene chloride (ABI); capping is performed with 16% *N*-methyl imidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); and oxidation solution is 16.9 mM I_2, 49 mM pyridine, 9% water in THF (PERSEPTIVE™). Burdick & Jackson Synthesis Grade acetonitrile is used directly from the reagent bottle. S-Ethyltetrazole solution (0.25 M in acetonitrile) is made up from the solid obtained from American International Chemical, Inc. Alternately, for the introduction of phosphorothioate linkages, Beaucage reagent (3H-1,2-Benzodithiol-3-one 1,1-dioxide, 0.05 M in acetonitrile) is used.

Deprotection of the antisense oligonucleotides is performed as follows: the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 40% aq. methylamine (1 mL) at 65 °C for 10 min. After cooling to –20 °C, the supernatant is removed from the polymer support. The support is washed three times with 1.0 mL of EtOH:MeCN:H2O/3:1:1, vortexed and the supernatant is then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, are dried to a white powder.

The method of synthesis used for normal RNA including certain enzymatic nucleic acid molecules follows the procedure as described in Usman *et al.*, 1987, *J. Am. Chem. Soc.*, 109, 7845; Scaringe *et al.*, 1990, *Nucleic Acids Res.*, 18, 5433; Wincott *et al.*, 1995, *Nucleic Acids Res.* 23, 2677-2684 and Wincott *et al.*, 1997, *Methods Mol. Bio.*, 74, 59, and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. In a non-limiting example, small scale syntheses are conducted on a 394 Applied Biosystems, Inc. synthesizer using a 0.2 μmol scale protocol with a 7.5 min coupling step for alkylsilyl protected nucleotides and a 2.5 min coupling step for 2'-O-methylated nucleotides. **Table II** outlines the amounts and the contact times of the reagents used in the synthesis cycle. Alternatively, syntheses at the 0.2 μmol scale can be done on a 96-well plate synthesizer, such as the instrument produced by Protogene (Palo Alto, CA) with minimal

modification to the cycle. A 33-fold excess (60 μL of 0.11 M = 6.6 μmol) of 2'-O-methyl phosphoramidite and a 75-fold excess of S-ethyl tetrazole (60 μL of 0.25 M = 15 μmol) can be used in each coupling cycle of 2'-O-methyl residues relative to polymer-bound 5'-hydroxyl. A 66-fold excess (120 μL of 0.11 M = 13.2 μmol) of alkylsilyl (ribo) protected phosphoramidite and a 150-fold excess of S-ethyl tetrazole (120 μL of 0.25 M = 30 μmol) can be used in each coupling cycle of ribo residues relative to polymer-bound 5'-hydroxyl. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by colorimetric quantitation of the trityl fractions, are typically 97.5-99%. Other oligonucleotide synthesis reagents for the 394 Applied Biosystems, Inc. synthesizer include; detritylation solution is 3% TCA in methylene chloride (ABI); capping is performed with 16% *N*-methyl imidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); oxidation solution is 16.9 mM I_2, 49 mM pyridine, 9% water in THF (PERSEPTIVE™). Burdick & Jackson Synthesis Grade acetonitrile is used directly from the reagent bottle. S-Ethyltetrazole solution (0.25 M in acetonitrile) is made up from the solid obtained from American International Chemical, Inc. Alternately, for the introduction of phosphorothioate linkages, Beaucage reagent (3H-1,2-Benzodithiol-3-one 1,1-dioxide0.05 M in acetonitrile) is used.

Deprotection of the RNA is performed using either a two-pot or one-pot protocol. For the two-pot protocol, the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 40% aq. methylamine (1 mL) at 65 °C for 10 min. After cooling to –20 °C, the supernatant is removed from the polymer support. The support is washed three times with 1.0 mL of EtOH:MeCN:H2O/3:1:1, vortexed and the supernatant is then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, are dried to a white powder. The base deprotected oligoribonucleotide is resuspended in anhydrous TEA/HF/NMP solution (300 μL of a solution of 1.5 mL N-methylpyrrolidinone, 750 μL TEA and 1 mL TEA•3HF to provide a 1.4 M HF concentration) and heated to 65 °C. After 1.5 h, the oligomer is quenched with 1.5 M NH_4HCO_3.

Alternatively, for the one-pot protocol, the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 33% ethanolic methylamine/DMSO: 1/1 (0.8 mL) at 65 °C for 15 min. The vial is brought to r.t. TEA•3HF (0.1 mL) is added and the vial is heated at 65 °C for 15 min. The sample is cooled at –20 °C and then quenched with 1.5 M NH_4HCO_3.

For purification of the trityl-on oligomers, the quenched NH_4HCO_3 solution is loaded onto a C-18 containing cartridge that had been prewashed with acetonitrile followed by 50 mM TEAA. After washing the loaded cartridge with water, the RNA is detritylated with 0.5% TFA

for 13 min. The cartridge is then washed again with water, salt exchanged with 1 M NaCl and washed with water again. The oligonucleotide is then eluted with 30% acetonitrile.

Inactive hammerhead ribozymes or binding attenuated control (BAC) oligonucleotides) are synthesized by substituting a U for G_5 and a U for A_{14} (numbering from Hertel, K. J., *et al.*, 1992, *Nucleic Acids Res.*, 20, 3252). Similarly, one or more nucleotide substitutions can be introduced in other enzymatic nucleic acid molecules to inactivate the molecule and such molecules can serve as a negative control.

The average stepwise coupling yields are typically >98% (Wincott *et al.*, 1995 *Nucleic Acids Res.* 23, 2677-2684). Those of ordinary skill in the art will recognize that the scale of synthesis can be adapted to be larger or smaller than the examples described above including but not limited to 96-well format, all that is important is the ratio of chemicals used in the reaction.

Alternatively, the nucleic acid molecules of the present invention can be synthesized separately and joined together post-synthetically, for example by ligation (Moore *et al.*, 1992, *Science* 256, 9923; Draper *et al.*, International PCT publication No. WO 93/23569; Shabarova *et al.*, 1991, *Nucleic Acids Research* 19, 4247; Bellon *et al.*, 1997, *Nucleosides & Nucleotides*, 16, 951; Bellon *et al.*, 1997, *Bioconjugate Chem.* 8, 204).

The nucleic acid molecules of the present invention are modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-*C*-allyl, 2'-flouro, 2'-*O*-methyl, 2'-H (for a review see Usman and Cedergren, 1992, *TIBS* 17, 34; Usman *et al.*, 1994, *Nucleic Acids Symp. Ser.* 31, 163). Ribozymes are purified by gel electrophoresis using general methods or are purified by high pressure liquid chromatography (HPLC; See Wincott *et al.*, *supra*, the totality of which is hereby incorporated herein by reference) and are re-suspended in water.

The sequences of the ribozymes and antisense constructs that are chemically synthesized, useful in this study, are shown in **Tables III to IX**. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. The ribozyme and antisense construct sequences listed in **Tables III to IX** may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes with enzymatic activity are equivalent to the ribozymes described specifically in the Tables.

<u>Optimizing Activity of the nucleic acid molecule of the invention.</u>

Chemically synthesizing nucleic acid molecules with modifications (base, sugar and/or phosphate) that prevent their degradation by serum ribonucleases can increase their potency (see

e.g., Eckstein *et al.*, International Publication No. WO 92/07065; Perrault *et al.*, 1990 *Nature* 344, 565; Pieken *et al.*, 1991, *Science* 253, 314; Usman and Cedergren, 1992, *Trends in Biochem. Sci.* 17, 334; Usman *et al.*, International Publication No. WO 93/15187; Rossi *et al.*, International Publication No. WO 91/03162; Sproat, US Patent No. 5,334,711; and Burgin *et al., supra)*; all of these describe various chemical modifications that can be made to the base, phosphate and/or sugar moieties of the nucleic acid molecules described herein. All these references are incorporated by reference herein. Modifications which enhance their efficacy in cells, and removal of bases from nucleic acid molecules to shorten oligonucleotide synthesis times and reduce chemical requirements are desired.

There are several examples in the art describing sugar, base and phosphate modifications that can be introduced into nucleic acid molecules with significant enhancement in their nuclease stability and efficacy. For example, oligonucleotides are modified to enhance stability and/or enhance biological activity by modification with nuclease resistant groups, for example, 2'-amino, 2'-*C*-allyl, 2'-flouro, 2'-*O*-methyl, 2'-H, nucleotide base modifications (for a review see Usman and Cedergren, 1992, *TIBS.* 17, 34; Usman *et al.*, 1994, *Nucleic Acids Symp. Ser.* 31, 163; Burgin *et al.*, 1996, *Biochemistry* , 35, 14090). Sugar modifications of nucleic acid molecules have been extensively described in the art (see Eckstein *et al.*, *International Publication* PCT No. WO 92/07065; Perrault *et al. Nature*, 1990, *344*, 565-568; Pieken *et al. Science,* 1991, *253*, 314-317; Usman and Cedergren, *Trends in Biochem. Sci.* , 1992, *17*, 334-339; Usman *et al. International Publication* PCT No. WO 93/15187; Sproat, US Patent No. 5,334,711 and Beigelman *et al.*, 1995, *J. Biol. Chem.*, 270, 25702; Beigelman *et al.*, International PCT publication No. WO 97/26270; Beigelman *et al.*, US Patent No. 5,716,824; Usman *et al.*, US patent No. 5,627,053; Woolf *et al.*, International PCT Publication No. WO 98/13526; Thompson *et al.*, USSN 60/082,404 which was filed on April 20, 1998; Karpeisky *et al.*, 1998, *Tetrahedron Lett.*, 39, 1131; Earnshaw and Gait, 1998, *Biopolymers (Nucleic acid Sciences)*, 48, 39-55; Verma and Eckstein, 1998, *Annu. Rev. Biochem.*, 67, 99-134; and Burlina *et al.*, 1997, *Bioorg. Med. Chem.*, 5, 1999-2010; all of the references are hereby incorporated by reference herein in their totalities). Such publications describe general methods and strategies to determine the location of incorporation of sugar, base and/or phosphate modifications and the like into ribozymes without inhibiting catalysis. In view of such teachings, similar modifications can be used as described herein to modify the nucleic acid molecules of the instant invention.

While chemical modification of oligonucleotide internucleotide linkages with phosphorothioate, phosphorothioate, and/or 5'-methylphosphonate linkages improves stability, too many of these modifications may cause some toxicity. Therefore when designing nucleic acid molecules the amount of these internucleotide linkages should be minimized. The reduction

in the concentration of these linkages should lower toxicity resulting in increased efficacy and higher specificity of these molecules.

Nucleic acid molecules having chemical modifications which maintain or enhance activity are provided. Such nucleic acid is also generally more resistant to nucleases than unmodified nucleic acid. Thus, in a cell and/or *in vivo* the activity may not be significantly lowered. Therapeutic nucleic acid molecules delivered exogenously must optimally be stable within cells until translation of the target RNA has been inhibited long enough to reduce the levels of the undesirable protein. This period of time varies between hours to days depending upon the disease state. Clearly, nucleic acid molecules must be resistant to nucleases in order to function as effective intracellular therapeutic agents. Improvements in the chemical synthesis of RNA and DNA (Wincott *et al.*, 1995 *Nucleic Acids Res.* 23, 2677; Caruthers *et al.*, 1992, *Methods in Enzymology* 211,3-19 (incorporated by reference herein) have expanded the ability to modify nucleic acid molecules by introducing nucleotide modifications to enhance their nuclease stability as described above.

Use of the nucleic acid-based molecules of the present invention will lead to better treatment of the disease progression by affording the possibility of combination therapies (*e.g.*, multiple antisense or enzymatic nucleic acid molecules targeted to different genes, nucleic acid molecules coupled with known small molecule inhibitors, or intermittent treatment with combinations of molecules (including different motifs) and/or other chemical or biological molecules). The treatment of patients with nucleic acid molecules can also include combinations of different types of nucleic acid molecules.

Therapeutic nucleic acid molecules (*e.g.*, enzymatic nucleic acid molecules and antisense nucleic acid molecules) delivered exogenously must optimally be stable within cells until translation of the target RNA has been inhibited long enough to reduce the levels of the undesirable protein. This period of time varies between hours to days depending upon the disease state. Clearly, these nucleic acid molecules must be resistant to nucleases in order to function as effective intracellular therapeutic agents. Improvements in the chemical synthesis of nucleic acid molecules described in the instant invention and in the art have expanded the ability to modify nucleic acid molecules by introducing nucleotide modifications to enhance their nuclease stability as described above.

In yet another preferred embodiment, nucleic acid catalysts having chemical modifications which maintain or enhance enzymatic activity are provided. Such nucleic acid is also generally more resistant to nucleases than unmodified nucleic acid. Thus, in a cell and/or *in vivo* the activity may not be significantly lowered. As exemplified herein such ribozymes are useful in a

cell and/or *in vivo* even if activity over all is reduced 10 fold (Burgin *et al.*, 1996, *Biochemistry*, 35, 14090). Such ribozymes herein are said to "maintain" the enzymatic activity of an all RNA ribozyme.

In another aspect the nucleic acid molecules comprise a 5' and/or a 3'- cap structure.

By "cap structure" is meant chemical modifications, which have been incorporated at either terminus of the oligonucleotide (see, for example, Wincott *et al.*, WO 97/26270, incorporated by reference herein). These terminal modifications protect the nucleic acid molecule from exonuclease degradation, and may help in delivery and/or localization within a cell. The cap may be present at the 5'-terminus (5'-cap) or at the 3'-terminus (3'-cap) or may be present on both termini. In non-limiting examples the 5'-cap is selected from the group comprising inverted abasic residue (moiety), 4',5'-methylene nucleotide; 1-(beta-D-erythrofuranosyl) nucleotide, 4'-thio nucleotide, carbocyclic nucleotide; 1,5-anhydrohexitol nucleotide; L-nucleotides; alpha-nucleotides; modified base nucleotide; phosphorodithioate linkage; *threo*-pentofuranosyl nucleotide; acyclic 3',4'-seco nucleotide; acyclic 3,4-dihydroxybutyl nucleotide; acyclic 3,5-dihydroxypentyl nucleotide, 3'-3'-inverted nucleotide moiety; 3'-3'-inverted abasic moiety; 3'-2'-inverted nucleotide moiety; 3'-2'-inverted abasic moiety; 1,4-butanediol phosphate; 3'-phosphoramidate; hexylphosphate; aminohexyl phosphate; 3'-phosphate; 3'-phosphorothioate; phosphorodithioate; or bridging or non-bridging methylphosphonate moiety (for more details see Wincott *et al.*, International PCT publication No. WO 97/26270, incorporated by reference herein).

In yet another preferred embodiment, the 3'-cap is selected from a group comprising, 4',5'-methylene nucleotide; 1-(beta-D-erythrofuranosyl) nucleotide; 4'-thio nucleotide, carbocyclic nucleotide; 5'-amino-alkyl phosphate; 1,3-diamino-2-propyl phosphate, 3-aminopropyl phosphate; 6-aminohexyl phosphate; 1,2-aminododecyl phosphate; hydroxypropyl phosphate; 1,5-anhydrohexitol nucleotide; L-nucleotide; alpha-nucleotide; modified base nucleotide; phosphorodithioate; *threo*-pentofuranosyl nucleotide; acyclic 3',4'-seco nucleotide; 3,4-dihydroxybutyl nucleotide; 3,5-dihydroxypentyl nucleotide, 5'-5'-inverted nucleotide moiety; 5'-5'-inverted abasic moiety; 5'-phosphoramidate; 5'-phosphorothioate; 1,4-butanediol phosphate; 5'-amino; bridging and/or non-bridging 5'-phosphoramidate, phosphorothioate and/or phosphorodithioate, bridging or non bridging methylphosphonate and 5'-mercapto moieties (for more details, see Beaucage and Iyer, 1993, *Tetrahedron* 49, 1925; incorporated by reference herein).

By the term "non-nucleotide" is meant any group or compound which can be incorporated into a nucleic acid chain in the place of one or more nucleotide units, including either sugar

and/or phosphate substitutions, and allows the remaining bases to exhibit their enzymatic activity. The group or compound is abasic in that it does not contain a commonly recognized nucleotide base, such as adenosine, guanine, cytosine, uracil or thymine.

An "alkyl" group refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. Preferably, the alkyl group has 1 to 12 carbons. More preferably it is a lower alkyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO2 or N(CH3)2, amino, or SH. The term also includes alkenyl groups which are unsaturated hydrocarbon groups containing at least one carbon-carbon double bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkenyl group has 1 to 12 carbons. More preferably it is a lower alkenyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkenyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO2, halogen, N(CH3)2, amino, or SH. The term "alkyl" also includes alkynyl groups which have an unsaturated hydrocarbon group containing at least one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkynyl group has 1 to 12 carbons. More preferably it is a lower alkynyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkynyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO2 or N(CH3)2, amino or SH.

Such alkyl groups may also include aryl, alkylaryl, carbocyclic aryl, heterocyclic aryl, amide and ester groups. An "aryl" group refers to an aromatic group which has at least one ring having a conjugated π electron system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted. The preferred substituent(s) of aryl groups are halogen, trihalomethyl, hydroxyl, SH, OH, cyano, alkoxy, alkyl, alkenyl, alkynyl, and amino groups. An "alkylaryl" group refers to an alkyl group (as described above) covalently joined to an aryl group (as described above). Carbocyclic aryl groups are groups wherein the ring atoms on the aromatic ring are all carbon atoms. The carbon atoms are optionally substituted. Heterocyclic aryl groups are groups having from 1 to 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms are carbon atoms. Suitable heteroatoms include oxygen, sulfur, and nitrogen, and include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted. An "amide" refers to an -C(O)-NH-R, where R is either alkyl, aryl, alkylaryl or hydrogen. An "ester" refers to an -C(O)-OR', where R is either alkyl, aryl, alkylaryl or hydrogen.

By "nucleotide" is meant a heterocyclic nitrogenous base in N-glycosidic linkage with a phosphorylated sugar. Nucleotides are recognized in the art to include natural bases (standard), and modified bases well known in the art. Such bases are generally located at the 1' position of a nucleotide sugar moiety. Nucleotides generally comprise a base, sugar and a phosphate group. The nucleotides can be unmodified or modified at the sugar, phosphate and/or base moiety, (also referred to interchangeably as nucleotide analogs, modified nucleotides, non-natural nucleotides, non-standard nucleotides and other; see for example, Usman and McSwiggen, supra; Eckstein et al., International PCT Publication No. WO 92/07065; Usman et al., International PCT Publication No. WO 93/15187; Uhlman & Peyman, supra all are hereby incorporated by reference herein). There are several examples of modified nucleic acid bases known in the art as summarized by Limbach et al., 1994, Nucleic Acids Res. 22, 2183. Some of the non-limiting examples of chemically modified and other natural nucleic acid bases that can be introduced into nucleic acids include, inosine, purine, pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2, 4, 6-trimethoxy benzene, 3-methyl uracil, dihydrouridine, naphthyl, aminophenyl, 5-alkylcytidines (e.g., 5-methylcytidine), 5-alkyluridines (e.g., ribothymidine), 5-halouridine (e.g., 5-bromouridine) or 6-azapyrimidines or 6-alkylpyrimidines (e.g. 6-methyluridine), propyne, quesosine, 2-thiouridine, 4-thiouridine, wybutosine, wybutoxosine, 4-acetylcytidine, 5-(carboxyhydroxymethyl)uridine, 5'-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluridine, beta-D-galactosylqueosine, 1-methyladenosine, 1-methylinosine, 2,2-dimethylguanosine, 3-methylcytidine, 2-methyladenosine, 2-methylguanosine, N6-methyladenosine, 7-methylguanosine, 5-methoxyaminomethyl-2-thiouridine, 5-methylaminomethyluridine, 5-methylcarbonylmethyluridine, 5-methyloxyuridine, 5-methyl-2-thiouridine, 2-methylthio-N6-isopentenyladenosine, -D-mannosylqueosine, uridine-5-oxyacetic acid, 2-thiocytidine, threonine derivatives and others (Burgin et al., 1996, Biochemistry, 35, 14090; Uhlman & Peyman, supra). By "modified bases" in this aspect is meant nucleotide bases other than adenine, guanine, cytosine and uracil at 1' position or their equivalents; such bases may be used at any position, for example, within the catalytic core of an enzymatic nucleic acid molecule and/or in the substrate-binding regions of the nucleic acid molecule.

By "nucleoside" is meant a heterocyclic nitrogenous base in N-glycosidic linkage with a sugar. Nucleosides are recognized in the art to include natural bases (standard), and modified bases well known in the art. Such bases are generally located at the 1' position of a nucleoside sugar moiety. Nucleosides generally comprise a base and sugar group. The nucleosides can be unmodified or modified at the sugar, and/or base moiety, (also referred to interchangeably as nucleoside analogs, modified nucleosides, non-natural nucleosides, non-standard nucleosides and other; see for example, Usman and McSwiggen, supra; Eckstein et al., International PCT

Publication No. WO 92/07065; Usman et al., International PCT Publication No. WO 93/15187; Uhlman & Peyman, supra all are hereby incorporated by reference herein). There are several examples of modified nucleic acid bases known in the art as summarized by Limbach et al., 1994, Nucleic Acids Res. 22, 2183. Some of the non-limiting examples of chemically modified and other natural nucleic acid bases that can be introduced into nucleic acids include, inosine, purine, pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2, 4, 6-trimethoxy benzene, 3-methyl uracil, dihydrouridine, naphthyl, aminophenyl, 5-alkylcytidines (e.g., 5-methylcytidine), 5-alkyluridines (e.g., ribothymidine), 5-halouridine (e.g., 5-bromouridine) or 6-azapyrimidines or 6-alkylpyrimidines (e.g. 6-methyluridine), propyne, quesosine, 2-thiouridine, 4-thiouridine, wybutosine, wybutoxosine, 4-acetylcytidine, 5-(carboxyhydroxymethyl)uridine, 5'-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluridine, -D-galactosylqueosine, 1-methyladenosine, 1-methylinosine, 2,2-dimethylguanosine, 3-methylcytidine, 2-methyladenosine, 2-methylguanosine, N6-methyladenosine, 7-methylguanosine, 5-methoxyaminomethyl-2-thiouridine, 5-methylaminomethyluridine, 5-methylcarbonylmethyluridine, 5-methyloxyuridine, 5-methyl-2-thiouridine, 2-methylthio-N6-isopentenyladenosine, beta-D-mannosylqueosine, uridine-5-oxyacetic acid, 2-thiocytidine, threonine derivatives and others (Burgin et al., 1996, Biochemistry, 35, 14090; Uhlman & Peyman, supra). By "modified bases" in this aspect is meant nucleoside bases other than adenine, guanine, cytosine and uracil at 1' position or their equivalents; such bases may be used at any position, for example, within the catalytic core of an enzymatic nucleic acid molecule and/or in the substrate-binding regions of the nucleic acid molecule.

In a preferred embodiment, the invention features modified ribozymes with phosphate backbone modifications comprising one or more phosphorothioate, phosphorodithioate, methylphosphonate, morpholino, amidate carbamate, carboxymethyl, acetamidate, polyamide, sulfonate, sulfonamide, sulfamate, formacetal, thioformacetal, and/or alkylsilyl, substitutions. For a review of oligonucleotide backbone modifications see Hunziker and Leumann, 1995, *Nucleic Acid Analogues: Synthesis and Properties,* in *Modern Synthetic Methods,* VCH, 331-417, and Mesmaeker *et al.,* 1994, *Novel Backbone Replacements for Oligonucleotides,* in *Carbohydrate Modifications in Antisense Research,* ACS, 24-39.

By "abasic" is meant sugar moieties lacking a base or having other chemical groups in place of a base at the 1' position, (for more details, see Wincott *et al.,* International PCT publication No. WO 97/26270).

By "unmodified nucleoside" is meant one of the bases adenine, cytosine, guanine, thymine, uracil joined to the 1' carbon of beta-D-ribo-furanose.

By "modified nucleoside" is meant any nucleotide base which contains a modification in the chemical structure of an unmodified nucleotide base, sugar and/or phosphate.

In connection with 2'-modified nucleotides as described for the present invention, by "amino" is meant 2'-NH_2 or 2'-*O*- NH_2, which may be modified or unmodified. Such modified groups are described, for example, in Eckstein *et al.*, U.S. Patent 5,672,695 and Matulic-Adamic *et al.*, WO 98/28317, respectively, which are both incorporated by reference herein in their entireties.

Various modifications to nucleic acid (*e.g.*, antisense and ribozyme) structure can be made to enhance the utility of these molecules. Such modifications will enhance shelf-life, half-life *in vitro*, stability, and ease of introduction of such oligonucleotides to the target site, *e.g.*, to enhance penetration of cellular membranes, and confer the ability to recognize and bind to targeted cells.

Use of these molecules will lead to better treatment of the disease progression by affording the possibility of combination therapies (e.g., multiple ribozymes targeted to different genes, ribozymes coupled with known small molecule inhibitors, or intermittent treatment with combinations of ribozymes (including different ribozyme motifs) and/or other chemical or biological molecules). The treatment of patients with nucleic acid molecules may also include combinations of different types of nucleic acid molecules. Therapies may be devised which include a mixture of ribozymes (including different ribozyme motifs), antisense and/or 2-5A chimera molecules to one or more targets to alleviate symptoms of a disease.

Administration of Nucleic Acid Molecules

Methods for the delivery of nucleic acid molecules are described in Akhtar *et al.*, 1992, *Trends Cell Bio.*, 2, 139; and *Delivery Strategies for Antisense Oligonucleotide Therapeutics*, ed. Akhtar, 1995 which are both incorporated herein by reference. Sullivan *et al.*, PCT WO 94/02595, further describes the general methods for delivery of enzymatic RNA molecules. These protocols may be utilized for the delivery of virtually any nucleic acid molecule. Nucleic acid molecules may be administered to cells by a variety of methods known to those familiar to the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres. For some indications, nucleic acid molecules may be directly delivered *ex vivo* to cells or tissues with or without the aforementioned vehicles. Alternatively, the nucleic acid/vehicle combination is locally delivered by direct injection or by use of a catheter, infusion pump or stent. Other routes of delivery include, but are not limited to, intravascular, intramuscular, subcutaneous or joint injection, aerosol inhalation, oral (tablet or

pill form), topical, systemic, ocular, intraperitoneal and/or intrathecal delivery. More detailed descriptions of nucleic acid delivery and administration are provided in Sullivan *et al.*, supra, Draper *et al.*, PCT WO93/23569, Beigelman *et al.*, PCT WO99/05094, and Klimuk *et al.*, PCT WO99/04819 all of which have been incorporated by reference herein.

The molecules of the instant invention can be used as pharmaceutical agents. Pharmaceutical agents prevent, inhibit the occurrence, or treat (alleviate a symptom to some extent, preferably all of the symptoms) of a disease state in a patient.

The negatively charged polynucleotides of the invention can be administered (*e.g.*, RNA, DNA or protein) and introduced into a patient by any standard means, with or without stabilizers, buffers, and the like, to form a pharmaceutical composition. When it is desired to use a liposome delivery mechanism, standard protocols for formation of liposomes can be followed. The compositions of the present invention may also be formulated and used as tablets, capsules or elixirs for oral administration; suppositories for rectal administration; sterile solutions; suspensions for injectable administration; and other compositions known in the art.

The present invention also includes pharmaceutically acceptable formulations of the compounds described. These formulations include salts of the above compounds, *e.g.*, acid addition salts, including salts of hydrochloric, hydrobromic, acetic acid, and benzene sulfonic acid.

A pharmacological composition or formulation refers to a composition or formulation in a form suitable for administration, *e.g.*, systemic administration, into a cell or patient, preferably a human. Suitable forms, in part, depend upon the use or the route of entry, for example oral, transdermal, or by injection. Such forms should not prevent the composition or formulation from reaching a target cell (*i.e.*, a cell to which the negatively charged polymer is desired to be delivered to). For example, pharmacological compositions injected into the blood stream should be soluble. Other factors are known in the art, and include considerations such as toxicity and forms which prevent the composition or formulation from exerting its effect.

By "systemic administration" is meant *in vivo* systemic absorption or accumulation of drugs in the blood stream followed by distribution throughout the entire body. Administration routes that lead to systemic absorption include, without limitations: intravenous, subcutaneous, intraperitoneal, inhalation, oral, intrapulmonary and intramuscular. Each of these administration routes exposes the desired negatively charged polymers, *e.g.*, nucleic acids, to an accessible diseased tissue. The rate of entry of a drug into the circulation has been shown to be a function of molecular weight or size. The use of a liposome or other drug carrier comprising the compounds of the instant invention can potentially localize the drug, for example, in certain

tissue types, such as the tissues of the reticular endothelial system (RES). A liposome formulation that can facilitate the association of drug with the surface of cells, such as, lymphocytes and macrophages is also useful. This approach may provide enhanced delivery of the drug to target cells by taking advantage of the specificity of macrophage and lymphocyte immune recognition of abnormal cells, such as cancer cells.

By pharmaceutically acceptable formulation is meant, a composition or formulation that allows for the effective distribution of the nucleic acid molecules of the instant invention in the physical location most suitable for their desired activity. Non-limiting examples of agents suitable for formulation with the nucleic acid molecules of the instant invention include: P-glycoprotein inhibitors (such as Pluronic P85) which can enhance entry of drugs into the CNS (Jolliet-Riant and Tillement, 1999, *Fundam. Clin. Pharmacol.*, 13, 16-26); biodegradable polymers, such as poly (DL-lactide-coglycolide) microspheres for sustained release delivery after intracerebral implantation (Emerich, DF *et al*, 1999, *Cell Transplant*, 8, 47-58) Alkermes, Inc. Cambridge, MA; and loaded nanoparticles, such as those made of polybutylcyanoacrylate, which can deliver drugs across the blood brain barrier and can alter neuronal uptake mechanisms (*Prog Neuropsychopharmacol Biol Psychiatry*, 23, 941-949, 1999). Other non-limiting examples of delivery strategies for the nucleic acid molecules of the instant invention include material described in Boado *et al.*, 1998, *J. Pharm. Sci.*, 87, 1308-1315; Tyler *et al.*, 1999, *FEBS Lett.*, 421, 280-284; Pardridge *et al.*, 1995, *PNAS USA.*, 92, 5592-5596; Boado, 1995, *Adv. Drug Delivery Rev.*, 15, 73-107; Aldrian-Herrada *et al.*, 1998, *Nucleic Acids Res.*, 26, 4910-4916; and Tyler *et al.*, 1999, *PNAS USA.*, 96, 7053-7058.

The invention also features the use of the composition comprising surface-modified liposomes containing poly (ethylene glycol) lipids (PEG-modified, or long-circulating liposomes or stealth liposomes). These formulations offer a method for increasing the accumulation of drugs in target tissues. This class of drug carriers resists opsonization and elimination by the mononuclear phagocytic system (MPS or RES), thereby enabling longer blood circulation times and enhanced tissue exposure for the encapsulated drug (Lasic *et al. Chem. Rev.* 1995, 95, 2601-2627; Ishiwata *et al.*, *Chem. Pharm. Bull.* 1995, 43, 1005-1011). All incorporated by reference herein. Such liposomes have been shown to accumulate selectively in tumors, presumably by extravasation and capture in the neovascularized target tissues (Lasic *et al., Science* 1995, 267, 1275-1276; Oku *et al.*,1995, *Biochim. Biophys. Acta*, 1238, 86-90). All incorporated by reference herein. The long-circulating liposomes enhance the pharmacokinetics and pharmacodynamics of DNA and RNA, particularly compared to conventional cationic liposomes which are known to accumulate in tissues of the MPS (Liu *et al., J. Biol. Chem.* 1995, 42, 24864-24870; Choi *et al.*, International PCT Publication No. WO 96/10391; Ansell *et al.*, International PCT Publication No. WO 96/10390; Holland *et al.*, International PCT Publication No. WO

96/10392; all of which are incorporated by reference herein). Long-circulating liposomes are also likely to protect drugs from nuclease degradation to a greater extent compared to cationic liposomes, based on their ability to avoid accumulation in metabolically aggressive MPS tissues such as the liver and spleen.

The present invention also includes compositions prepared for storage or administration which include a pharmaceutically effective amount of the desired compounds in a pharmaceutically acceptable carrier or diluent. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in *Remington's Pharmaceutical Sciences*, Mack Publishing Co. (A.R. Gennaro edit. 1985) hereby incorporated by reference herein. For example, preservatives, stabilizers, dyes and flavoring agents may be provided. These include sodium benzoate, sorbic acid and esters of *p*-hydroxybenzoic acid. In addition, antioxidants and suspending agents may be used.

A pharmaceutically effective dose is that dose required to prevent, inhibit the occurrence, or treat (alleviate a symptom to some extent, preferably all of the symptoms) of a disease state. The pharmaceutically effective dose depends on the type of disease, the composition used, the route of administration, the type of mammal being treated, the physical characteristics of the specific mammal under consideration, concurrent medication, and other factors which those skilled in the medical arts will recognize. Generally, an amount between 0.1 mg/kg and 100 mg/kg body weight/day of active ingredients is administered dependent upon potency of the negatively charged polymer.

The nucleic acid molecules of the present invention may also be administered to a patient in combination with other therapeutic compounds to increase the overall therapeutic effect. The use of multiple compounds to treat an indication may increase the beneficial effects while reducing the presence of side effects.

Alternatively, certain of the nucleic acid molecules of the instant invention can be expressed within cells from eukaryotic promoters (*e.g.*, Izant and Weintraub, 1985, *Science,* 229, 345; McGarry and Lindquist, 1986, *Proc. Natl. Acad. Sci.*, USA 83, 399; Scanlon *et al.,* 1991, *Proc. Natl. Acad. Sci. USA*, 88, 10591-5; Kashani-Sabet *et al.,* 1992, *Antisense Res. Dev.,* 2, 3-15; Dropulic *et al.,* 1992, *J. Virol.,* 66, 1432-41; Weerasinghe *et al.,* 1991, *J. Virol.,* 65, 5531-4; Ojwang *et al.,* 1992, *Proc. Natl. Acad. Sci. USA*, 89, 10802-6; Chen *et al.,* 1992, *Nucleic Acids Res.,* 20, 4581-9; Sarver *et al.,* 1990 *Science,* 247, 1222-1225; Thompson *et al.,* 1995, *Nucleic Acids Res.,* 23, 2259; Good *et al.,* 1997, *Gene Therapy*, 4, 45; all of the references are hereby incorporated in their totality by reference herein). Those skilled in the art realize that any nucleic acid can be expressed in eukaryotic cells from the appropriate DNA/RNA vector. The activity of

such nucleic acids can be augmented by their release from the primary transcript by a ribozyme (Draper *et al.*, PCT WO 93/23569, and Sullivan *et al.*, PCT WO 94/02595; Ohkawa *et al.*, 1992, *Nucleic Acids Symp. Ser.*, 27, 15-6; Taira *et al.*, 1991, *Nucleic Acids Res.*, 19, 5125-30; Ventura *et al.*, 1993, *Nucleic Acids Res.*, 21, 3249-55; Chowrira *et al.*, 1994, *J. Biol. Chem.*, 269, 25856; all of these references are hereby incorporated in their totalities by reference herein).

In another aspect of the invention, RNA molecules of the present invention are preferably expressed from transcription units (see, for example, Couture *et al.*, 1996, *TIG.*, 12, 510) inserted into DNA or RNA vectors. The recombinant vectors are preferably DNA plasmids or viral vectors. Ribozyme expressing viral vectors could be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. Preferably, the recombinant vectors capable of expressing the nucleic acid molecules are delivered as described above, and persist in target cells. Alternatively, viral vectors may be used that provide for transient expression of nucleic acid molecules. Such vectors might be repeatedly administered as necessary. Once expressed, the nucleic acid molecule binds to the target mRNA. Delivery of nucleic acid molecule expressing vectors could be systemic, such as by intravenous or intra-muscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that would allow for introduction into the desired target cell (for a review, see Couture *et al.*, 1996, *TIG.*, 12, 510).

In one aspect, the invention features an expression vector comprising a nucleic acid sequence encoding at least one of the nucleic acid molecules disclosed in the instant invention. The nucleic acid sequence encoding the nucleic acid molecule of the instant invention is operable linked in a manner which allows expression of that nucleic acid molecule.

In another aspect, the invention features an expression vector comprising: a) a transcription initiation region (*e.g.*, eukaryotic pol I, II or III initiation region); b) a transcription termination region (*e.g.*, eukaryotic pol I, II or III termination region); c) a nucleic acid sequence encoding at least one of the nucleic acid catalyst of the instant invention; and wherein said sequence is operably linked to said initiation region and said termination region, in a manner which allows expression and/or delivery of said nucleic acid molecule. The vector may optionally include an open reading frame (ORF) for a protein operably linked on the 5' side or the 3'-side of the sequence encoding the nucleic acid catalyst of the invention; and/or an intron (intervening sequences).

Transcription of the nucleic acid molecule sequences are driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters will be expressed at high levels in all cells; the

levels of a given pol II promoter in a given cell type will depend on the nature of the gene regulatory sequences (enhancers, silencers, etc.) present nearby. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Elroy-Stein and Moss, 1990, *Proc. Natl. Acad. Sci. U S A*, 87, 6743-7; Gao and Huang 1993, *Nucleic Acids Res..*, 21, 2867-72; Lieber *et al.*, 1993, *Methods Enzymol.*, 217, 47-66; Zhou *et al.*, 1990, *Mol. Cell. Biol.*, 10, 4529-37). All of these references are incorporated by reference herein.

Several investigators have demonstrated that nucleic acid molecules, such as ribozymes expressed from such promoters can function in mammalian cells (e.g. Kashani-Sabet *et al.*, 1992, *Antisense Res. Dev.*, 2, 3-15; Ojwang *et al.*, 1992, *Proc. Natl. Acad. Sci. U S A*, 89, 10802-6; Chen *et al.*, 1992, *Nucleic Acids Res.*, 20, 4581-9; Yu *et al.*, 1993, *Proc. Natl. Acad. Sci. U S A*, 90, 6340-4; L'Huillier *et al.*, 1992, *EMBO J.*, 11, 4411-8; Lisziewicz *et al.*, 1993, *Proc. Natl. Acad. Sci. U. S. A*, 90, 8000-4; Thompson *et al.*, 1995, *Nucleic Acids Res.*, 23, 2259; and Sullenger & Cech, 1993, *Science*, 262, 1566). More specifically, transcription units such as the ones derived from genes encoding U6 small nuclear (snRNA), transfer RNA (tRNA) and adenovirus VA RNA are useful in generating high concentrations of desired RNA molecules such as ribozymes in cells (Thompson *et al., supra*; Couture and Stinchcomb, *1996, supra*; Noonberg *et al.*, 1994, *Nucleic Acid Res.*, 22, 2830; Noonberg *et al.*, US Patent No. 5,624,803; Good *et al.*, 1997, *Gene Ther.*, 4, 45; and Beigelman *et al.*, International PCT Publication No. WO 96/18736; all of these publications are incorporated by reference herein. The above ribozyme transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as adenovirus or adeno-associated virus vectors), or viral RNA vectors (such as retroviral or alphavirus vectors) (for a review, see Couture and Stinchcomb, *1996, supra)*.

In yet another aspect, the invention features an expression vector comprising a nucleic acid sequence encoding at least one of the nucleic acid molecules of the invention, in a manner which allows expression of that nucleic acid molecule. The expression vector comprises in one embodiment; a) a transcription initiation region; b) a transcription termination region; c) a nucleic acid sequence encoding at least one said nucleic acid molecule; and wherein said sequence is operably linked to said initiation region and said termination region, in a manner which allows expression and/or delivery of said nucleic acid molecule.

In another preferred embodiment, the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an open reading frame; d) a nucleic acid sequence encoding at least one said nucleic acid molecule, wherein said sequence is operably linked to the 3'-end of said open reading frame; and wherein said sequence is operably

linked to said initiation region, said open reading frame and said termination region, in a manner which allows expression and/or delivery of said nucleic acid molecule.

In yet another embodiment the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an intron; d) a nucleic acid sequence encoding at least one said nucleic acid molecule; and wherein said sequence is operably linked to said initiation region, said intron and said termination region, in a manner which allows expression and/or delivery of said nucleic acid molecule.

In another embodiment, the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an intron; d) an open reading frame; e) a nucleic acid sequence encoding at least one said nucleic acid molecule, wherein said sequence is operably linked to the 3'-end of said open reading frame; and wherein said sequence is operably linked to said initiation region, said intron, said open reading frame and said termination region, in a manner which allows expression and/or delivery of said nucleic acid molecule.

Examples.

The following are non-limiting examples showing the selection, isolation, synthesis and activity of nucleic acids of the instant invention.

The following examples demonstrate the selection and design of Antisense, hammerhead, DNAzyme, NCH, Amberzyme, Zinzyme, or G-Cleaver ribozyme molecules and binding/cleavage sites within Chk1 RNA.

Nucleic acid inhibition of Chk1 target RNA

Control of the cell cycle is one of the most highly orchestrated events in the cell. There is a great deal of interest in discovering the function of genes involved in mitotic checkpoint abrogation, since inhibition of these genes or activities of these gene products could sensitize cells to DNA damaging agents. In these studies, the cell cycle regulatory role of Chk1 (GeneBank Accession # AF016582 is investigated).

In the fission yeast *Schizosaccharomyces pombe*, DNA damage by gamma irradiation or a chemical agent such as etoposide leads to activation of Chk1 by phosphorylation. Chk1, also known as p56chk1, is a Wee 1-like protein kinase, which phosphorylates and inactivates Cdc25. Cdc25 is a phosphatase that acts directly on Cdc2. Chk1 is required for the DNA damage checkpoint, whereas the rad gene products are required for both S-M and DNA damage checkpoints. Wee 1 is also phosphorylated by Chk1 *in vitro*, also suggesting that Wee 1 is regulated by Chk1 *in vivo* and the resulting G2 delay is the result of maintaining Y15

phosphorylation on Cdc2. In normal mammalian cells, DNA damage would lead to arrest at G1/S arrest *via* the p53 pathway, or G2/M arrest *via* the Cdc2/CyclinB pathway. Thus, p53- cells can remain viable following DNA damage because of the Cdc2/CyclinB arrest pathway. If the Cdc2/CyclinB mediated checkpoint is abrogated *via* inhibition of Wee1 and Myt1 by small molecule inhibitors in a p53- cell type, then viability is compromised. Chk1 has recently been cloned from mammalian cells. The Chk1 protein is modified in response to DNA damage, and has been shown to bind and phosphorylate Cdc25A, Cdc25B and Cdc25C. The phosphorylation of Cdc25C prevents activation of the Cdc2/CyclinB complex and blocks entry into mitosis, thereby validating the inhibition of Chk1 as a target for nucleic acid based therapeutics.

To address whether checkpoint kinases function redundantly during DNA replication and/or DNA damage checkpoint responses, applicant undertook an oligonucleotide-based approach to block Chk1 gene function in a human cell line. HeLa cells lacking Chk1 protein failed to maintain a G2 cell cycle arrest after etoposide or gamma radiation-induced DNA damaging treatments. Additionally, Chk1-defeicient cells failed to respond to the DNA replication inhibitor hydroxyurea. Based on these results, applicant concludes that the Chk1 kinase plays an essential role in both the DNA replication and DNA damage checkpoint responses. These results also suggest the neither Chk2 nor C-TAK1 kinases function in these checkpoint responses to a significant level, at least in HeLa cells. Thus, Chk1 is validated as an attractive therapeutic target for abrogating the G2 DNA damage checkpoint arrest; a situation that may selectively sensitize p53-deficient tumor cells to radiation or chemotherapy treatment.

Example 1: Identification of Potential Target Sites in Human Chk1 RNA

The sequence of human Chk1 is screened for accessible sites using a computer-folding algorithm. Regions of the RNA are identified that do not form secondary folding structures. These regions contain potential ribozyme and/or antisense binding/cleavage sites. The sequences of these binding/cleavage sites are shown in **Tables III-IX.**

Example 2: Selection of Enzymatic Nucleic Acid Cleavage Sites in Human Chk1 RNA

Ribozyme target sites are chosen by analyzing sequences of Human Chk1 (Genbank accession number: AF016582) and prioritizing the sites on the basis of folding. Ribozymes are designed that could bind each target and are individually analyzed by computer folding (Christoffersen *et al.*, 1994 *J. Mol. Struc. Theochem,* 311, 273; Jaeger *et al.*, 1989, *Proc. Natl. Acad. Sci. USA*, 86, 7706) to assess whether the ribozyme sequences fold into the appropriate secondary structure. Those ribozymes with unfavorable intramolecular interactions between the binding arms and the catalytic core are eliminated from consideration. As noted below, varying

binding arm lengths can be chosen to optimize activity. Generally, at least 5 bases on each arm are able to bind to, or otherwise interact with, the target RNA.

Example 3: Chemical Synthesis and Purification of Ribozymes and Antisense for Efficient Cleavage and/or blocking of Chk1 RNA

Ribozymes and antisense constructs are designed to anneal to various sites in the RNA message. The binding arms of the ribozymes are complementary to the target site sequences described above, while the antisense constructs are fully complimentary to the target site sequences described above. The ribozymes and antisense constructs were chemically synthesized. The method of synthesis used followed the procedure for normal RNA synthesis as described above and in Usman *et al.*, (1987 *J. Am. Chem. Soc.*, 109, 7845), Scaringe *et al.*, (1990 *Nucleic Acids Res.*, 18, 5433) and Wincott *et al.*, *supra*, and made use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The average stepwise coupling yields were typically >98%.

Ribozymes and antisense constructs are also synthesized from DNA templates using bacteriophage T7 RNA polymerase (Milligan and Uhlenbeck, 1989, *Methods Enzymol.* 180, 51). Ribozymes and antisense constructs are purified by gel electrophoresis using general methods or are purified by high pressure liquid chromatography (HPLC; see Wincott *et al.*, *supra*; the totality of which is hereby incorporated herein by reference) and are resuspended in water. The sequences of the chemically synthesized ribozymes and antisense constructs used in this study are shown below in **Table III-IX.**

Example 4: Ribozyme Cleavage of Chk1 RNA Target *in vitro*

Ribozymes targeted to the human Chk1 RNA are designed and synthesized as described above. These ribozymes can be tested for cleavage activity *in vitro*, for example, using the following procedure. The target sequences and the nucleotide location within the Chk1 RNA are given in **Tables III-IX.**

Cleavage Reactions: Full-length or partially full-length, internally-labeled target RNA for ribozyme cleavage assay is prepared by *in vitro* transcription in the presence of [a-^{32}p] CTP, passed over a G 50 Sephadex® column by spin chromatography and used as substrate RNA without further purification. Alternately, substrates are 5'-^{32}P-end labeled using T4 polynucleotide kinase enzyme. Assays are performed by pre-warming a 2X concentration of purified ribozyme in ribozyme cleavage buffer (50 mM Tris-HCl, pH 7.5 at 37°C, 10 mM $MgCl_2$) and the cleavage reaction was initiated by adding the 2X ribozyme mix to an equal volume of substrate RNA (maximum of 1-5 nM) that was also pre-warmed in cleavage buffer.

As an initial screen, assays are carried out for 1 hour at 37°C using a final concentration of either 40 nM or 1 mM ribozyme, *i.e.*, ribozyme excess. The reaction is quenched by the addition of an equal volume of 95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol after which the sample is heated to 95°C for 2 minutes, quick chilled and loaded onto a denaturing polyacrylamide gel. Substrate RNA and the specific RNA cleavage products generated by ribozyme cleavage are visualized on an autoradiograph of the gel. The percentage of cleavage is determined by Phosphor Imager® quantitation of bands representing the intact substrate and the cleavage products.

Example 5: Nucleic acid inhibition of Chk1 target RNA *in vivo*

Antisense nucleic acid molecules (GeneBlocs™) targeted to the human Chk1 RNA are designed and synthesized as described above. These nucleic acid molecules can be tested for cleavage activity *in vivo*, for example, using the following procedure. The target sequences and the nucleotide location within the Chk1 RNA are given in **Tables III-IX**.

Two formats were used to test the efficacy of nucleic acid reagents (GeneBlocs™ targeting Chk1. First, the reagents were tested on asynchronous HeLa cells, to determine the extent of RNA and protein inhibition. To demonstrate whether cells bypass the G2/M checkpoint, HeLa cells (p53-) are treated with etoposide to damage the DNA. Nocodazole and the potential checkpoint inhibitor are added 16 hours later, when all the cells should be arrested in G2. Nocodazole blocks cells from leaving mitosis, so if they have abrogated the checkpoint, the cells will be blocked in mitosis and appear "rounded" in shape. Other surrogate mitotic markers include decreased phosphorylation of cdc-2 at Thr14 and Tyr15, phosphorylation of Myt-1, and phosphorylation of PP1. This study set out to determine whether inhibiting expression of the Chk1 gene would allow the G2/M checkpoint to be bypassed after DNA damage, as well as determining if the presence of p53 influences the DNA-damage checkpoint response.

Eight GeneBloc™ reagents (e.g.; see **Table IX**) were selected against the Chk1 cDNA target. RNA inhibition was measured after delivery of these reagents by GSV lipid (Glenn Research) to HeLa cells. Relative amounts of target RNA were measured versus actin using real-time PCR monitoring of amplification (ABI 7700 Taqman®). The results are shown in **Figure 6**. The comparison is made to a mixture of 5 oligonucleotide sequences made to unrelated targets (GB-3) or to a randomized oligonucleotide control with the same overall length and chemistry, but randomly substituted at each position (GBC3.2). Primary and secondary lead reagents were chosen for the target and optimization performed. The optimal GSV lipid concentration was chosen after screening for RNA inhibition with oligonucleotides at 5 and 50 nM (**Figure 7**). After optimal lipid concentration was chosen, a RNA time-course of inhibition

was performed with the lead nucleic acid molecule (GeneBloc™) (**Figure 8**). In addition, a cell-plating format was tested for RNA inhibition. The use of a 96-well (5000 cells/well) versus six-well (150,000 cells/well) plating density made no difference in the extent of RNA inhibition (**Figure 9**). The phenotypic assays require treatment with etoposide and nocodazole as described above, and RNA inhibition in this assay was also determined (**Figure 10**). The various treatments had essentially no effect on RNA levels.

Optimization of delivery conditions were also performed in DLD-1 (p53-) (**Figure 11**) and MCF-7 (**Figure 12**) (p53+) cells. Similar levels of inhibition were observed when compared to HeLa cells at the optimal GSV concentration. Dose curves were also generated in HeLa cells with the two best lead nucleic acid molecules (**Figure 13**). IC50 values for both leads were in the 1-2 nM range. Similar IC50s were observed in DLD-1 and MCF-7 cells. Protein levels were assessed at 8, 24 and 32 hours after nucleic acid administration, as well as one to five days post delivery. The target protein was significantly reduced (80-90%) by 24 hours after nucleic acid administration and remained low (undetectable by western blot) until at least day 5. Application of nucleic acid inhibitors in the checkpoint abrogation assay resulted in the "rounding up" phenotype for the Chk1 target. Also, there is an increase in Myt 1 phosphorylation and a large increase in PP1 phosphorylation. There also appear to be decreases in phosphorylation of the Y15 and T14 residues on Cdc2, although this is not complete. Most importantly, this evidence demonstrates the role of Chk1 in the G2/M checkpoint and suggests that inhibitors of Chk1 activity can be useful alone or in combination with DNA damaging agents in treatment of certain types of cancer.

<u>Indications</u>

Particular degenerative and disease states that can be associated with Chk1 expression modulation include but are not limited to cancers of the colon, rectum, lung, breast and prostate

The present body of knowledge in Chk1 research indicates the need for methods to assay Chk1 activity and for compounds that can regulate Chk1 expression for research, diagnostic, and therapeutic use.

Radiation and chemotherapeutic treatments are non-limiting examples of methods that can be combined with or used in conjunction with the nucleic acid molecules (*e.g.* ribozymes and antisense molecules) of the instant invention. Those skilled in the art will recognize that other drug compounds and therapies can be similarly be readily combined with the nucleic acid molecules of the instant invention (*e.g.* ribozymes and antisense molecules) are hence within the scope of the instant invention.

Diagnostic uses

The nucleic acid molecules of this invention (*e.g., ribozymes)* can be used as diagnostic tools to examine genetic drift and mutations within diseased cells or to detect the presence of Chk1 RNA in a cell. The close relationship between ribozyme activity and the structure of the target RNA allows the detection of mutations in any region of the molecule which alters the base-pairing and three-dimensional structure of the target RNA. By using multiple ribozymes described in this invention, one can map nucleotide changes which are important to RNA structure and function *in vitro*, as well as in cells and tissues. Cleavage of target RNAs with ribozymes can be used to inhibit gene expression and define the role (essentially) of specified gene products in the progression of disease. In this manner, other genetic targets can be defined as important mediators of the disease. These experiments will lead to better treatment of the disease progression by affording the possibility of combinational therapies (*e.g.*, multiple ribozymes targeted to different genes, ribozymes coupled with known small molecule inhibitors, or intermittent treatment with combinations of ribozymes and/or other chemical or biological molecules). Other *in vitro* uses of ribozymes of this invention are well known in the art, and include detection of the presence of mRNAs associated with Chk1-related condition. Such RNA is detected by determining the presence of a cleavage product after treatment with a ribozyme using standard methodology.

In a specific example, ribozymes which can cleave only wild-type or mutant forms of the target RNA are used for the assay. The first ribozyme is used to identify wild-type RNA present in the sample and the second ribozyme is used to identify mutant RNA in the sample. As reaction controls, synthetic substrates of both wild-type and mutant RNA is cleaved by both ribozymes to demonstrate the relative ribozyme efficiencies in the reactions and the absence of cleavage of the "non-targeted" RNA species. The cleavage products from the synthetic substrates also serve to generate size markers for the analysis of wild-type and mutant RNAs in the sample population. Thus, each analysis requires two ribozymes, two substrates and one unknown sample, which is combined into six reactions. The presence of cleavage products is determined using an RNAse protection assay so that full-length and cleavage fragments of each RNA can be analyzed in one lane of a polyacrylamide gel. It is not absolutely required to quantify the results to gain insight into the expression of mutant RNAs and putative risk of the desired phenotypic changes in target cells. The expression of mRNA whose protein product is implicated in the development of the phenotype (*i.e.*, Chk1) is adequate to establish risk. If probes of comparable specific activity are used for both transcripts, then a qualitative comparison of RNA levels will be adequate and will decrease the cost of the initial diagnosis. Higher mutant form to wild-type ratios are correlated with higher risk whether RNA levels are compared qualitatively or quantitatively.

Additional Uses

Potential usefulness of sequence-specific enzymatic nucleic acid molecules of the instant invention might have many of the same applications for the study of RNA that DNA restriction endonucleases have for the study of DNA (Nathans *et al.*, 1975 *Ann. Rev. Biochem.* 44:273). For example, the pattern of restriction fragments can be used to establish sequence relationships between two related RNAs, and large RNAs could be specifically cleaved to fragments of a size more useful for study. The ability to engineer sequence specificity of the enzymatic nucleic acid molecule is ideal for cleavage of RNAs of unknown sequence. Applicant has described the use of nucleic acid molecules to down-regulate gene expression of target genes in bacterial, microbial, fungal, viral, and eukaryotic systems including plant, or mammalian cells.

All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in the art to which the invention pertains. All references cited in this disclosure are incorporated by reference to the same extent as if each reference had been incorporated by reference in its entirety individually.

One skilled in the art would readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The methods and compositions described herein as presently representative of preferred embodiments are exemplary and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art, which are encompassed within the spirit of the invention, are defined by the scope of the claims.

It will be readily apparent to one skilled in the art that varying substitutions and modifications can be made to the invention disclosed herein without departing from the scope and spirit of the invention. Thus, such additional embodiments are within the scope of the present invention and the following claims.

The invention illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of" and "consisting of" may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments, optional features, modification and variation of the concepts herein disclosed may

be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the description and the appended claims.

In addition, where features or aspects of the invention are described in terms of Markush groups or other grouping of alternatives, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group or other group.

Other embodiments are within the claims that follow.

TABLE I

Characteristics of naturally occurring ribozymes

Group I Introns

- Size: ~150 to >1000 nucleotides.
- Requires a U in the target sequence immediately 5' of the cleavage site.
- Binds 4-6 nucleotides at the 5'-side of the cleavage site.
- Reaction mechanism: attack by the 3′-OH of guanosine to generate cleavage products with 3′-OH and 5′-guanosine.
- Additional protein cofactors required in some cases to help folding and maintainance of the active structure.
- Over 300 known members of this class. Found as an intervening sequence in *Tetrahymena thermophila* rRNA, fungal mitochondria, chloroplasts, phage T4, blue-green algae, and others.
- Major structural features largely established through phylogenetic comparisons, mutagenesis, and biochemical studies [i,ii].
- Complete kinetic framework established for one ribozyme [iii,iv,v,vi].
- Studies of ribozyme folding and substrate docking underway [vii,viii,ix].
- Chemical modification investigation of important residues well established [x,xi].
- The small (4-6 nt) binding site may make this ribozyme too non-specific for targeted RNA cleavage, however, the Tetrahymena group I intron has been used to repair a "defective" beta-galactosidase message by the ligation of new beta-galactosidase sequences onto the defective message [xii].

RNAse P RNA (M1 RNA)

- Size: ~290 to 400 nucleotides.
- RNA portion of a ubiquitous ribonucleoprotein enzyme.
- Cleaves tRNA precursors to form mature tRNA [xiii].
- Reaction mechanism: possible attack by M^{2+}-OH to generate cleavage products with 3′-OH and 5′-phosphate.

- RNAse P is found throughout the prokaryotes and eukaryotes. The RNA subunit has been sequenced from bacteria, yeast, rodents, and primates.
- Recruitment of endogenous RNAse P for therapeutic applications is possible through hybridization of an External Guide Sequence (EGS) to the target RNA [xiv,xv]
- Important phosphate and 2′ OH contacts recently identified [xvi,xvii]

Group II Introns

- Size: >1000 nucleotides.
- Trans cleavage of target RNAs recently demonstrated [xviii,xix].
- Sequence requirements not fully determined.
- Reaction mechanism: 2′-OH of an internal adenosine generates cleavage products with 3′-OH and a "lariat" RNA containing a 3′-5′ and a 2′-5′ branch point.
- Only natural ribozyme with demonstrated participation in DNA cleavage [xx,xxi] in addition to RNA cleavage and ligation.
- Major structural features largely established through phylogenetic comparisons [xxii].
- Important 2′ OH contacts beginning to be identified [xxiii]
- Kinetic framework under development [xxiv]

Neurospora VS RNA

- Size: ~144 nucleotides.
- Trans cleavage of hairpin target RNAs recently demonstrated [xxv].
- Sequence requirements not fully determined.
- Reaction mechanism: attack by 2′-OH 5′ to the scissile bond to generate cleavage products with 2′,3′-cyclic phosphate and 5′-OH ends.
- Binding sites and structural requirements not fully determined.
- Only 1 known member of this class. Found in Neurospora VS RNA.

Hammerhead Ribozyme

(see text for references)

- Size: ~13 to 40 nucleotides.
- Requires the target sequence UH immediately 5' of the cleavage site.
- Binds a variable number nucleotides on both sides of the cleavage site.
- Reaction mechanism: attack by 2′-OH 5′ to the scissile bond to generate cleavage products with 2′,3′-cyclic phosphate and 5′-OH ends.
- 14 known members of this class. Found in a number of plant pathogens (virusoids) that use RNA as the infectious agent.
- Essential structural features largely defined, including 2 crystal structures [xxvi,xxvii]
- Minimal ligation activity demonstrated (for engineering through *in vitro* selection) [xxviii]
- Complete kinetic framework established for two or more ribozymes [xxix].
- Chemical modification investigation of important residues well established [xxx].

Hairpin Ribozyme

- Size: ~50 nucleotides.
- Requires the target sequence GUC immediately 3' of the cleavage site.
- Binds 4-6 nucleotides at the 5'-side of the cleavage site and a variable number to the 3'-side of the cleavage site.
- Reaction mechanism: attack by 2′-OH 5′ to the scissile bond to generate cleavage products with 2′,3′-cyclic phosphate and 5′-OH ends.
- 3 known members of this class. Found in three plant pathogen (satellite RNAs of the tobacco ringspot virus, arabis mosaic virus and chicory yellow mottle virus) which uses RNA as the infectious agent.
- Essential structural features largely defined [xxxi,xxxii,xxxiii,xxxiv]
- Ligation activity (in addition to cleavage activity) makes ribozyme amenable to engineering through *in vitro* selection [xxxv]
- Complete kinetic framework established for one ribozyme [xxxvi].
- Chemical modification investigation of important residues begun [xxxvii,xxxviii].

Hepatitis Delta Virus (HDV) Ribozyme

- Size: ~60 nucleotides.
- Trans cleavage of target RNAs demonstrated [xxxix].
- Binding sites and structural requirements not fully determined, although no sequences 5' of cleavage site are required. Folded ribozyme contains a pseudoknot structure [xl].
- Reaction mechanism: attack by 2'-OH 5' to the scissile bond to generate cleavage products with 2',3'-cyclic phosphate and 5'-OH ends.
- Only 2 known members of this class. Found in human HDV.
- Circular form of HDV is active and shows increased nuclease stability [xli]

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Table II:

A. 2.5 µmol Synthesis Cycle ABI 394 Instrument

Reagent	Equivalents	Amount	Wait Time* DNA	Wait Time* 2'-O-methyl	Wait Time*RNA
Phosphoramidites	6.5	163 µL	45 sec	2.5 min	7.5 min
S-Ethyl Tetrazole	23.8	238 µL	45 sec	2.5 min	7.5 min
Acetic Anhydride	100	233 µL	5 sec	5 sec	5 sec
N-Methyl Imidazole	186	233 µL	5 sec	5 sec	5 sec
TCA	176	2.3 mL	21 sec	21 sec	21 sec
Iodine	11.2	1.7 mL	45 sec	45 sec	45 sec
Beaucage	12.9	645 µL	100 sec	300 sec	300 sec
Acetonitrile	NA	6.67 mL	NA	NA	NA

B. 0.2 µmol Synthesis Cycle ABI 394 Instrument

Reagent	Equivalents	Amount	Wait Time* DNA	Wait Time* 2'-O-methyl	Wait Time*RNA
Phosphoramidites	15	31 µL	45 sec	233 sec	465 sec
S-Ethyl Tetrazole	38.7	31 µL	45 sec	233 min	465 sec
Acetic Anhydride	655	124 µL	5 sec	5 sec	5 sec
N-Methyl Imidazole	1245	124 µL	5 sec	5 sec	5 sec
TCA	700	732 µL	10 sec	10 sec	10 sec
Iodine	20.6	244 µL	15 sec	15 sec	15 sec
Beaucage	7.7	232 µL	100 sec	300 sec	300 sec
Acetonitrile	NA	2.64 mL	NA	NA	NA

C. 0.2 µmol Synthesis Cycle 96 well Instrument

Reagent	Equivalents:DNA/ 2'-O-methyl/Ribo	Amount: DNA/2'-O-methyl/Ribo	Wait Time* DNA	Wait Time* 2'-O-methyl	Wait Time* Ribo
Phosphoramidites	22/33/66	40/60/120 µL	60 sec	180 sec	360sec
S-Ethyl Tetrazole	70/105/210	40/60/120 µL	60 sec	180 min	360 sec
Acetic Anhydride	265/265/265	50/50/50 µL	10 sec	10 sec	10 sec
N-Methyl Imidazole	502/502/502	50/50/50 µL	10 sec	10 sec	10 sec
TCA	238/475/475	250/500/500 µL	15 sec	15 sec	15 sec
Iodine	6.8/6.8/6.8	80/80/80 µL	30 sec	30 sec	30 sec
Beaucage	34/51/51	80/120/120	100 sec	200 sec	200 sec
Acetonitrile	NA	1150/1150/1150 µL	NA	NA	NA

- **Wait time does not include contact time during delivery.**

Table III: Human Chk1 Hammerhead Ribozyme and Substrate Sequence

Pos	Substrate	Seq ID	Ribozyme	Rz Seq ID
12	CGGACAGU C CGCCGAGG	1	CCUCGGCG CUGAUGAG GCCGUUAGGC CGAA ACUGUCCG	1423
25	GAGGUGCU C GGUGGAGU	2	ACUCCACC CUGAUGAG GCCGUUAGGC CGAA AGCACCUC	1424
34	GGUGGAGU C AUGGCAGU	3	ACUGCCAU CUGAUGAG GCCGUUAGGC CGAA ACUCCACC	1425
48	AGUGCCCU U UGUGGAAG	4	CUUCCACA CUGAUGAG GCCGUUAGGC CGAA AGGGCACU	1426
49	GUGCCCUU U GUGGAAGA	5	UCUUCCAC CUGAUGAG GCCGUUAGGC CGAA AAGGGCAC	1427
66	CUGGGACU U GGUGCAAA	6	UUUGCACC CUGAUGAG GCCGUUAGGC CGAA AGUCCCAG	1428
93	AGGUGCCU A UGGAGAAG	7	CUUCUCCA CUGAUGAG GCCGUUAGGC CGAA AGGCACCU	1429
103	GGAGAAGU U CAACUUGC	8	GCAAGUUG CUGAUGAG GCCGUUAGGC CGAA ACUUCUCC	1430
104	GAGAAGUU C AACUUGCU	9	AGCAAGUU CUGAUGAG GCCGUUAGGC CGAA AACUUCUC	1431
109	GUUCAACU U GCUGUGAA	10	UUCACAGC CUGAUGAG GCCGUUAGGC CGAA AGUUGAAC	1432
119	CUGUGAAU A GAGUAACU	11	AGUUACUC CUGAUGAG GCCGUUAGGC CGAA AUUCACAG	1433
124	AAUAGAGU A ACUGAAGA	12	UCUUCAGU CUGAUGAG GCCGUUAGGC CGAA ACUCUAUU	1434
139	GAAGCAGU C GCAGUGAA	13	UUCACUGC CUGAUGAG GCCGUUAGGC CGAA ACUGCUUC	1435
151	GUGAAGAU U GUAGAUAU	14	AUAUCUAC CUGAUGAG GCCGUUAGGC CGAA AUCUUCAC	1436
154	AAGAUUGU A GAUAUGAA	15	UUCAUAUC CUGAUGAG GCCGUUAGGC CGAA ACAAUCUU	1437
158	UUGUAGAU A UGAAGCGU	16	ACGCUUCA CUGAUGAG GCCGUUAGGC CGAA AUCUACAA	1438
172	CGUGCCGU A GACUGUCC	17	GGACAGUC CUGAUGAG GCCGUUAGGC CGAA ACGGCACG	1439
179	UAGACUGU C CAGAAAAU	18	AUUUUCUG CUGAUGAG GCCGUUAGGC CGAA ACAGUCUA	1440
188	CAGAAAAU A UUAAGAAA	19	UUUCUUAA CUGAUGAG GCCGUUAGGC CGAA AUUUUCUG	1441
190	GAAAAUAU U AAGAAAGA	20	UCUUUCUU CUGAUGAG GCCGUUAGGC CGAA AUAUUUUC	1442
191	AAAAUAUU A AGAAAGAG	21	CUCUUUCU CUGAUGAG GCCGUUAGGC CGAA AAUAUUUU	1443
202	AAAGAGAU C UGUAUCAA	22	UUGAUACA CUGAUGAG GCCGUUAGGC CGAA AUCUCUUU	1444
206	AGAUCUGU A UCAAUAAA	23	UUUAUUGA CUGAUGAG GCCGUUAGGC CGAA ACAGAUCU	1445
208	AUCUGUAU C AAUAAAAU	24	AUUUUAUU CUGAUGAG GCCGUUAGGC CGAA AUACAGAU	1446
212	GUAUCAAU A AAAUGCUA	25	UAGCAUUU CUGAUGAG GCCGUUAGGC CGAA AUUGAUAC	1447
220	AAAAUGCU A AAUCAUGA	26	UCAUGAUU CUGAUGAG GCCGUUAGGC CGAA AGCAUUUU	1448
224	UGCUAAAU C AUGAAAAU	27	AUUUUCAU CUGAUGAG GCCGUUAGGC CGAA AUUUAGCA	1449
235	GAAAAUGU A GUAAAAUU	28	AAUUUUAC CUGAUGAG GCCGUUAGGC CGAA ACAUUUUC	1450
238	AAUGUAGU A AAAUUCUA	29	UAGAAUUU CUGAUGAG GCCGUUAGGC CGAA ACUACAUU	1451
243	AGUAAAAU U CUAUGGUC	30	GACCAUAG CUGAUGAG GCCGUUAGGC CGAA AUUUUACU	1452
244	GUAAAAUU C UAUGGUCA	31	UGACCAUA CUGAUGAG GCCGUUAGGC CGAA AAUUUUAC	1453
246	AAAAUUCU A UGGUCACA	32	UGUGACCA CUGAUGAG GCCGUUAGGC CGAA AGAAUUUU	1454
251	UCUAUGGU C ACAGGAGA	33	UCUCCUGU CUGAUGAG GCCGUUAGGC CGAA ACCAUAGA	1455
269	AAGGCAAU A UCCAAUAU	34	AUAUUGGA CUGAUGAG GCCGUUAGGC CGAA AUUGCCUU	1456
271	GGCAAUAU C CAAUAUUU	35	AAAUAUUG CUGAUGAG GCCGUUAGGC CGAA AUAUUGCC	1457
276	UAUCCAAU A UUUAUUUC	36	GAAAUAAA CUGAUGAG GCCGUUAGGC CGAA AUUGGAUA	1458
278	UCCAAUAU U UAUUUCUG	37	CAGAAAUA CUGAUGAG GCCGUUAGGC CGAA AUAUUGGA	1459
279	CCAAUAUU U AUUUCUGG	38	CCAGAAAU CUGAUGAG GCCGUUAGGC CGAA AAUAUUGG	1460
280	CAAUAUUU A UUUCUGGA	39	UCCAGAAA CUGAUGAG GCCGUUAGGC CGAA AAAUAUUG	1461
282	AUAUUUAU U UCUGGAGU	40	ACUCCAGA CUGAUGAG GCCGUUAGGC CGAA AUAAAUAU	1462
283	UAUUUAUU U CUGGAGUA	41	UACUCCAG CUGAUGAG GCCGUUAGGC CGAA AAUAAAUA	1463
284	AUUUAUUU C UGGAGUAC	42	GUACUCCA CUGAUGAG GCCGUUAGGC CGAA AAAUAAAU	1464
291	UCUGGAGU A CUGUAGUG	43	CACUACAG CUGAUGAG GCCGUUAGGC CGAA ACUCCAGA	1465
296	AGUACUGU A GUGGAGGA	44	UCCUCCAC CUGAUGAG GCCGUUAGGC CGAA ACAGUACU	1466
310	GGAGAGCU U UUUGACAG	45	CUGUCAAA CUGAUGAG GCCGUUAGGC CGAA AGCUCUCC	1467
311	GAGAGCUU U UUGACAGA	46	UCUGUCAA CUGAUGAG GCCGUUAGGC CGAA AAGCUCUC	1468

312	AGAGCUUU U UGACAGAA	47	UUCUGUCA CUGAUGAG GCCGUUAGGC CGAA AAAGCUCU	1469
313	GAGCUUUU U GACAGAAU	48	AUUCUGUC CUGAUGAG GCCGUUAGGC CGAA AAAAGCUC	1470
322	GACAGAAU A GAGCCAGA	49	UCUGGCUC CUGAUGAG GCCGUUAGGC CGAA AUUCUGUC	1471
334	CCAGACAU A GGCAUGCC	50	GGCAUGCC CUGAUGAG GCCGUUAGGC CGAA AUGUCUGG	1472
356	CAGAUGCU C AGAGAUUC	51	GAAUCUCU CUGAUGAG GCCGUUAGGC CGAA AGCAUCUG	1473
363	UCAGAGAU U CUUCCAUC	52	GAUGGAAG CUGAUGAG GCCGUUAGGC CGAA AUCUCUGA	1474
364	CAGAGAUU C UUCCAUCA	53	UGAUGGAA CUGAUGAG GCCGUUAGGC CGAA AAUCUCUG	1475
366	GAGAUUCU U CCAUCAAC	54	GUUGAUGG CUGAUGAG GCCGUUAGGC CGAA AGAAUCUC	1476
367	AGAUUCUU C CAUCAACU	55	AGUUGAUG CUGAUGAG GCCGUUAGGC CGAA AAGAAUCU	1477
371	UCUUCCAU C AACUCAUG	56	CAUGAGUU CUGAUGAG GCCGUUAGGC CGAA AUGGAAGA	1478
376	CAUCAACU C AUGGCAGG	57	CCUGCCAU CUGAUGAG GCCGUUAGGC CGAA AGUUGAUG	1479
391	GGGGUGGU U UAUCUGCA	58	UGCAGAUA CUGAUGAG GCCGUUAGGC CGAA ACCACCCC	1480
392	GGGUGGUU U AUCUGCAU	59	AUGCAGAU CUGAUGAG GCCGUUAGGC CGAA AACCACCC	1481
393	GGUGGUUU A UCUGCAUG	60	CAUGCAGA CUGAUGAG GCCGUUAGGC CGAA AAACCACC	1482
395	UGGUUUAU C UGCAUGGU	61	ACCAUGCA CUGAUGAG GCCGUUAGGC CGAA AUAAACCA	1483
404	UGCAUGGU A UUGGAAUA	62	UAUUCCAA CUGAUGAG GCCGUUAGGC CGAA ACCAUGCA	1484
406	CAUGGUAU U GGAAUAAC	63	GUUAUUCC CUGAUGAG GCCGUUAGGC CGAA AUACCAUG	1485
412	AUUGGAAU A ACUCACAG	64	CUGUGAGU CUGAUGAG GCCGUUAGGC CGAA AUUCCAAU	1486
416	GAAUAACU C ACAGGGAU	65	AUCCCUGU CUGAUGAG GCCGUUAGGC CGAA AGUUAUUC	1487
425	ACAGGGAU A UUAAACCA	66	UGGUUUAA CUGAUGAG GCCGUUAGGC CGAA AUCCCUGU	1488
427	AGGGAUAU U AAACCAGA	67	UCUGGUUU CUGAUGAG GCCGUUAGGC CGAA AUAUCCCU	1489
428	GGGAUAUU A AACCAGAA	68	UUCUGGUU CUGAUGAG GCCGUUAGGC CGAA AAUAUCCC	1490
440	CAGAAAAU C UUCUGUUG	69	CAACAGAA CUGAUGAG GCCGUUAGGC CGAA AUUUUCUG	1491
442	GAAAAUCU U CUGUUGGA	70	UCCAACAG CUGAUGAG GCCGUUAGGC CGAA AGAUUUUC	1492
443	AAAAUCUU C UGUUGGAU	71	AUCCAACA CUGAUGAG GCCGUUAGGC CGAA AAGAUUUU	1493
447	UCUUCUGU U GGAUGAAA	72	UUUCAUCC CUGAUGAG GCCGUUAGGC CGAA ACAGAAGA	1494
461	AAAGGGAU A ACCUCAAA	73	UUUGAGGU CUGAUGAG GCCGUUAGGC CGAA AUCCCUUU	1495
466	GAUAACCU C AAAAUCUC	74	GAGAUUUU CUGAUGAG GCCGUUAGGC CGAA AGGUUAUC	1496
472	CUCAAAAU C UCAGACUU	75	AAGUCUGA CUGAUGAG GCCGUUAGGC CGAA AUUUUGAG	1497
474	CAAAAUCU C AGACUUUG	76	CAAAGUCU CUGAUGAG GCCGUUAGGC CGAA AGAUUUUG	1498
480	CUCAGACU U UGGCUUGG	77	CCAAGCCA CUGAUGAG GCCGUUAGGC CGAA AGUCUGAG	1499
481	UCAGACUU U GGCUUGGC	78	GCCAAGCC CUGAUGAG GCCGUUAGGC CGAA AAGUCUGA	1500
486	CUUUGGCU U GGCAACAG	79	CUGUUGCC CUGAUGAG GCCGUUAGGC CGAA AGCCAAAG	1501
496	GCAACAGU A UUUCGGUA	80	UACCGAAA CUGAUGAG GCCGUUAGGC CGAA ACUGUUGC	1502
498	AACAGUAU U UCGGUAUA	81	UAUACCGA CUGAUGAG GCCGUUAGGC CGAA AUACUGUU	1503
499	ACAGUAUU U CGGUAUAA	82	UUAUACCG CUGAUGAG GCCGUUAGGC CGAA AAUACUGU	1504
500	CAGUAUUU C GGUAUAAU	83	AUUAUACC CUGAUGAG GCCGUUAGGC CGAA AAAUACUG	1505
504	AUUUCGGU A UAAUAAUC	84	GAUUAUUA CUGAUGAG GCCGUUAGGC CGAA ACCGAAAU	1506
506	UUCGGUAU A AUAAUCGU	85	ACGAUUAU CUGAUGAG GCCGUUAGGC CGAA AUACCGAA	1507
509	GGUAUAAU A AUCGUGAG	86	CUCACGAU CUGAUGAG GCCGUUAGGC CGAA AUUAUACC	1508
512	AUAAUAAU C GUGAGCGU	87	ACGCUCAC CUGAUGAG GCCGUUAGGC CGAA AUUAUUAU	1509
521	GUGAGCGU U UGUUGAAC	88	GUUCAACA CUGAUGAG GCCGUUAGGC CGAA ACGCUCAC	1510
522	UGAGCGUU U GUUGAACA	89	UGUUCAAC CUGAUGAG GCCGUUAGGC CGAA AACGCUCA	1511
525	GCGUUUGU U GAACAAGA	90	UCUUGUUC CUGAUGAG GCCGUUAGGC CGAA ACAAACGC	1512
542	UGUGUGGU A CUUUACCA	91	UGGUAAAG CUGAUGAG GCCGUUAGGC CGAA ACCACACA	1513
545	GUGGUACU U UACCAUAU	92	AUAUGGUA CUGAUGAG GCCGUUAGGC CGAA AGUACCAC	1514
546	UGGUACUU U ACCAUAUG	93	CAUAUGGU CUGAUGAG GCCGUUAGGC CGAA AAGUACCA	1515
547	GGUACUUU A CCAUAUGU	94	ACAUAUGG CUGAUGAG GCCGUUAGGC CGAA AAAGUACC	1516
552	UUUACCAU A UGUUGCUC	95	GAGCAACA CUGAUGAG GCCGUUAGGC CGAA AUGGUAAA	1517

556	CCAUAUGU U GCUCCAGA	96	UCUGGAGC CUGAUGAG GCCGUUAGGC CGAA ACAUAUGG	1518
560	AUGUUGCU C CAGAACUU	97	AAGUUCUG CUGAUGAG GCCGUUAGGC CGAA AGCAACAU	1519
568	CCAGAACU U CUGAAGAG	98	CUCUUCAG CUGAUGAG GCCGUUAGGC CGAA AGUUCUGG	1520
569	CAGAACUU C UGAAGAGA	99	UCUCUUCA CUGAUGAG GCCGUUAGGC CGAA AAGUUCUG	1521
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586	AGAGAAUU U CAUGCAGA	101	UCUGCAUG CUGAUGAG GCCGUUAGGC CGAA AAUUCUCU	1523
587	GAGAAUUU C AUGCAGAA	102	UUCUGCAU CUGAUGAG GCCGUUAGGC CGAA AAAUUCUC	1524
601	GAACCAGU U GAUGUUUG	103	CAAACAUC CUGAUGAG GCCGUUAGGC CGAA ACUGGUUC	1525
607	GUUGAUGU U UGGUCCUG	104	CAGGACCA CUGAUGAG GCCGUUAGGC CGAA ACAUCAAC	1526
608	UUGAUGUU U GGUCCUGU	105	ACAGGACC CUGAUGAG GCCGUUAGGC CGAA AACAUCAA	1527
612	UGUUUGGU C CUGUGGAA	106	UUCCACAG CUGAUGAG GCCGUUAGGC CGAA ACCAAACA	1528
622	UGUGGAAU A GUACUUAC	107	GUAAGUAC CUGAUGAG GCCGUUAGGC CGAA AUUCCACA	1529
625	GGAAUAGU A CUUACUGC	108	GCAGUAAG CUGAUGAG GCCGUUAGGC CGAA ACUAUUCC	1530
628	AUAGUACU U ACUGCAAU	109	AUUGCAGU CUGAUGAG GCCGUUAGGC CGAA AGUACUAU	1531
629	UAGUACUU A CUGCAAUG	110	CAUUGCAG CUGAUGAG GCCGUUAGGC CGAA AAGUACUA	1532
640	GCAAUGCU C GCUGGAGA	111	UCUCCAGC CUGAUGAG GCCGUUAGGC CGAA AGCAUUGC	1533
651	UGGAGAAU U GCCAUGGG	112	CCCAUGGC CUGAUGAG GCCGUUAGGC CGAA AUUCUCCA	1534
680	ACAGCUGU C AGGAGUAU	113	AUACUCCU CUGAUGAG GCCGUUAGGC CGAA ACAGCUGU	1535
687	UCAGGAGU A UUCUGACU	114	AGUCAGAA CUGAUGAG GCCGUUAGGC CGAA ACUCCUGA	1536
689	AGGAGUAU U CUGACUGG	115	CCAGUCAG CUGAUGAG GCCGUUAGGC CGAA AUACUCCU	1537
690	GGAGUAUU C UGACUGGA	116	UCCAGUCA CUGAUGAG GCCGUUAGGC CGAA AAUACUCC	1538
714	AAAAACAU A CCUCAACC	117	GGUUGAGG CUGAUGAG GCCGUUAGGC CGAA AUGUUUUU	1539
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725	UCAACCCU U GGAAAAAA	119	UUUUUUCC CUGAUGAG GCCGUUAGGC CGAA AGGGUUGA	1541
736	AAAAAAAU C GAUUCUGC	120	GCAGAAUC CUGAUGAG GCCGUUAGGC CGAA AUUUUUUU	1542
740	AAAUCGAU U CUGCUCCU	121	AGGAGCAG CUGAUGAG GCCGUUAGGC CGAA AUCGAUUU	1543
741	AAUCGAUU C UGCUCCUC	122	GAGGAGCA CUGAUGAG GCCGUUAGGC CGAA AAUCGAUU	1544
746	AUUCUGCU C CUCUAGCU	123	AGCUAGAG CUGAUGAG GCCGUUAGGC CGAA AGCAGAAU	1545
749	CUGCUCCU C UAGCUCUG	124	CAGAGCUA CUGAUGAG GCCGUUAGGC CGAA AGGAGCAG	1546
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764	UGCUGCAU A AAAUCUUA	127	UAAGAUUU CUGAUGAG GCCGUUAGGC CGAA AUGCAGCA	1549
769	CAUAAAAU C UUAGUUGA	128	UCAACUAA CUGAUGAG GCCGUUAGGC CGAA AUUUUAUG	1550
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775	AUCUUAGU U GAGAAUCC	131	GGAUUCUC CUGAUGAG GCCGUUAGGC CGAA ACUAAGAU	1553
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797	CAAGAAUU A CCAUUCCA	135	UGGAAUGG CUGAUGAG GCCGUUAGGC CGAA AAUUCUUG	1557
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803	UUACCAUU C CAGACAUC	137	GAUGUCUG CUGAUGAG GCCGUUAGGC CGAA AAUGGUAA	1559
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821	AAAAAGAU A GAUGGUAC	139	GUACCAUC CUGAUGAG GCCGUUAGGC CGAA AUCUUUUU	1561
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868	CCCCGAGU C ACUUCAGG	142	CCUGAAGU CUGAUGAG GCCGUUAGGC CGAA ACUCGGGG	1564
872	GAGUCACU U CAGGUGGU	143	ACCACCUG CUGAUGAG GCCGUUAGGC CGAA AGUGACUC	1565
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893	CAGAGUCU C CCAGUGGA	147	UCCACUGG CUGAUGAG GCCGUUAGGC CGAA AGACUCUG	1569
903	CAGUGGAU U UUCUAAGC	148	GCUUAGAA CUGAUGAG GCCGUUAGGC CGAA AUCCACUG	1570
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906	UGGAUUUU C UAAGCACA	151	UGUGCUUA CUGAUGAG GCCGUUAGGC CGAA AAAAUCCA	1573
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917	AGCACAUU C AAUCCAAU	154	AUUGGAUU CUGAUGAG GCCGUUAGGC CGAA AAUGUGCU	1576
921	CAUUCAAU C CAAUUUGG	155	CCAAAUUG CUGAUGAG GCCGUUAGGC CGAA AUUGAAUG	1577
926	AAUCCAAU U UGGACUUC	156	GAAGUCCA CUGAUGAG GCCGUUAGGC CGAA AUUGGAUU	1578
927	AUCCAAUU U GGACUUCU	157	AGAAGUCC CUGAUGAG GCCGUUAGGC CGAA AAUUGGAU	1579
933	UUUGGACU U CUCUCCAG	158	CUGGAGAG CUGAUGAG GCCGUUAGGC CGAA AGUCCAAA	1580
934	UUGGACUU C UCUCCAGU	159	ACUGGAGA CUGAUGAG GCCGUUAGGC CGAA AAGUCCAA	1581
936	GGACUUCU C UCCAGUAA	160	UUACUGGA CUGAUGAG GCCGUUAGGC CGAA AGAAGUCC	1582
938	ACUUCUCU C CAGUAAAC	161	GUUUACUG CUGAUGAG GCCGUUAGGC CGAA AGAGAAGU	1583
943	UCUCCAGU A AACAGUGC	162	GCACUGUU CUGAUGAG GCCGUUAGGC CGAA ACUGGAGA	1584
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954	CAGUGCUU C UAGUGAAG	164	CUUCACUA CUGAUGAG GCCGUUAGGC CGAA AAGCACUG	1586
956	GUGCUUCU A GUGAAGAA	165	UUCUUCAC CUGAUGAG GCCGUUAGGC CGAA AGAAGCAC	1587
975	UGUGAAGU A CUCCAGUU	166	AACUGGAG CUGAUGAG GCCGUUAGGC CGAA ACUUCACA	1588
978	GAAGUACU C CAGUUCUC	167	GAGAACUG CUGAUGAG GCCGUUAGGC CGAA AGUACUUC	1589
983	ACUCCAGU U CUCAGCCA	168	UGGCUGAG CUGAUGAG GCCGUUAGGC CGAA ACUGGAGU	1590
984	CUCCAGUU C UCAGCCAG	169	CUGGCUGA CUGAUGAG GCCGUUAGGC CGAA AACUGGAG	1591
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1010	CAGGUCUU U CCUUAUGG	173	CCAUAAGG CUGAUGAG GCCGUUAGGC CGAA AAGACCUG	1595
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1014	UCUUUCCU U AUGGGAUA	175	UAUCCCAU CUGAUGAG GCCGUUAGGC CGAA AGGAAAGA	1597
1015	CUUUCCUU A UGGGAUAC	176	GUAUCCCA CUGAUGAG GCCGUUAGGC CGAA AAGGAAAG	1598
1022	UAUGGGAU A CCAGCCCC	177	GGGGCUGG CUGAUGAG GCCGUUAGGC CGAA AUCCCAUA	1599
1032	CAGCCCCU C AUACAUUG	178	CAAUGUAU CUGAUGAG GCCGUUAGGC CGAA AGGGGCUG	1600
1035	CCCCUCAU A CAUUGAUA	179	UAUCAAUG CUGAUGAG GCCGUUAGGC CGAA AUGAGGGG	1601
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1043	ACAUUGAU A AAUUGGUA	181	UACCAAUU CUGAUGAG GCCGUUAGGC CGAA AUCAAUGU	1603
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1106	UGAAUAGU C AGUUACUU	196	AAGUAACU CUGAUGAG GCCGUUAGGC CGAA ACUAUUCA	1618
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1114	CAGUUACU U GGCACCCC	199	GGGGUGCC CUGAUGAG GCCGUUAGGC CGAA AGUAACUG	1621
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1278	UACUAUAU C AACAACUG	225	CAGUUGUU CUGAUGAG GCCGUUAGGC CGAA AUAUAGUA	1647
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1539	GAGAAGAU U AUCCUGUC	276	GACAGGAU CUGAUGAG GCCGUUAGGC CGAA AUCUUCUC	1698
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1570	UAGUAGUU C CUGAAGUG	283	CACUUCAG CUGAUGAG GCCGUUAGGC CGAA AACUACUA	1705
1580	UGAAGUGU U CACUUCCC	284	GGGAAGUG CUGAUGAG GCCGUUAGGC CGAA ACACUUCA	1706
1581	GAAGUGUU C ACUUCCCU	285	AGGGAAGU CUGAUGAG GCCGUUAGGC CGAA AACACUUC	1707
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1607	AACAUCUU C CAAUUUAU	294	AUAAAUUG CUGAUGAG GCCGUUAGGC CGAA AAGAUGUU	1716
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1616	CAAUUUAU U UUGUUUGU	298	ACAAACAA CUGAUGAG GCCGUUAGGC CGAA AUAAAUUG	1720
1617	AAUUUAUU U UGUUUGUU	299	AACAAACA CUGAUGAG GCCGUUAGGC CGAA AAUAAAUU	1721
1618	AUUUAUUU U GUUUGUUC	300	GAACAAAC CUGAUGAG GCCGUUAGGC CGAA AAAUAAAU	1722
1621	UAUUUUGU U UGUUCGGC	301	GCCGAACA CUGAUGAG GCCGUUAGGC CGAA ACAAAAUA	1723
1622	AUUUUGUU U GUUCGGCA	302	UGCCGAAC CUGAUGAG GCCGUUAGGC CGAA AACAAAAU	1724
1625	UUGUUUGU U CGGCAUAC	303	GUAUGCCG CUGAUGAG GCCGUUAGGC CGAA ACAAACAA	1725
1626	UGUUUGUU C GGCAUACA	304	UGUAUGCC CUGAUGAG GCCGUUAGGC CGAA AACAAACA	1726
1632	UUCGGCAU A CAAAUAAU	305	AUUAUUUG CUGAUGAG GCCGUUAGGC CGAA AUGCCGAA	1727
1638	AUACAAAU A AUACCUAU	306	AUAGGUAU CUGAUGAG GCCGUUAGGC CGAA AUUUGUAU	1728
1641	CAAAUAAU A CCUAUAUC	307	GAUAUAGG CUGAUGAG GCCGUUAGGC CGAA AUUAUUUG	1729
1645	UAAUACCU A UAUCUUAA	308	UUAAGAUA CUGAUGAG GCCGUUAGGC CGAA AGGUAUUA	1730
1647	AUACCUAU A UCUUAAUU	309	AAUUAAGA CUGAUGAG GCCGUUAGGC CGAA AUAGGUAU	1731
1649	ACCUAUAU C UUAAUUGU	310	ACAAUUAA CUGAUGAG GCCGUUAGGC CGAA AUAUAGGU	1732
1651	CUAUAUCU U AAUUGUAA	311	UUACAAUU CUGAUGAG GCCGUUAGGC CGAA AGAUAUAG	1733
1652	UAUAUCUU A AUUGUAAG	312	CUUACAAU CUGAUGAG GCCGUUAGGC CGAA AAGAUAUA	1734
1655	AUCUUAAU U GUAAGCAA	313	UUGCUUAC CUGAUGAG GCCGUUAGGC CGAA AUUAAGAU	1735
1658	UUAAUUGU A AGCAAAAC	314	GUUUUGCU CUGAUGAG GCCGUUAGGC CGAA ACAAUUAA	1736
1668	GCAAAACU U UGGGGAAA	315	UUUCCCCA CUGAUGAG GCCGUUAGGC CGAA AGUUUUGC	1737
1669	CAAAACUU U GGGGAAAG	316	CUUUCCCC CUGAUGAG GCCGUUAGGC CGAA AAGUUUUG	1738
1685	GGAUGAAU A GAAUUCAU	317	AUGAAUUC CUGAUGAG GCCGUUAGGC CGAA AUUCAUCC	1739
1690	AAUAGAAU U CAUUUGAU	318	AUCAAAUG CUGAUGAG GCCGUUAGGC CGAA AUUCUAUU	1740
1691	AUAGAAUU C AUUUGAUU	319	AAUCAAAU CUGAUGAG GCCGUUAGGC CGAA AAUUCUAU	1741
1694	GAAUUCAU U UGAUUAUU	320	AAUAAUCA CUGAUGAG GCCGUUAGGC CGAA AUGAAUUC	1742
1695	AAUUCAUU U GAUUAUUU	321	AAAUAAUC CUGAUGAG GCCGUUAGGC CGAA AAUGAAUU	1743
1699	CAUUUGAU U AUUUCUUC	322	GAAGAAAU CUGAUGAG GCCGUUAGGC CGAA AUCAAAUG	1744
1700	AUUUGAUU A UUUCUUCA	323	UGAAGAAA CUGAUGAG GCCGUUAGGC CGAA AAUCAAAU	1745
1702	UUGAUUAU U UCUUCAUG	324	CAUGAAGA CUGAUGAG GCCGUUAGGC CGAA AUAAUCAA	1746
1703	UGAUUAUU U CUUCAUGU	325	ACAUGAAG CUGAUGAG GCCGUUAGGC CGAA AAUAAUCA	1747
1704	GAUUAUUU C UUCAUGUG	326	CACAUGAA CUGAUGAG GCCGUUAGGC CGAA AAAUAAUC	1748
1706	UUAUUUCU U CAUGUGUG	327	CACACAUG CUGAUGAG GCCGUUAGGC CGAA AGAAAUAA	1749
1707	UAUUUCUU C AUGUGUGU	328	ACACACAU CUGAUGAG GCCGUUAGGC CGAA AAGAAAUA	1750
1716	AUGUGUGU U UAGUAUCU	329	AGAUACUA CUGAUGAG GCCGUUAGGC CGAA ACACACAU	1751
1717	UGUGUGUU U AGUAUCUG	330	CAGAUACU CUGAUGAG GCCGUUAGGC CGAA AACACACA	1752
1718	GUGUGUUU A GUAUCUGA	331	UCAGAUAC CUGAUGAG GCCGUUAGGC CGAA AAACACAC	1753
1721	UGUUUAGU A UCUGAAUU	332	AAUUCAGA CUGAUGAG GCCGUUAGGC CGAA ACUAAACA	1754
1723	UUUAGUAU C UGAAUUUG	333	CAAAUUCA CUGAUGAG GCCGUUAGGC CGAA AUACUAAA	1755
1729	AUCUGAAU U UGAAACUC	334	GAGUUUCA CUGAUGAG GCCGUUAGGC CGAA AUUCAGAU	1756
1730	UCUGAAUU U GAAACUCA	335	UGAGUUUC CUGAUGAG GCCGUUAGGC CGAA AAUUCAGA	1757
1737	UUGAAACU C AUCUGGUG	336	CACCAGAU CUGAUGAG GCCGUUAGGC CGAA AGUUUCAA	1758
1740	AAACUCAU C UGGUGGAA	337	UUCCACCA CUGAUGAG GCCGUUAGGC CGAA AUGAGUUU	1759
1756	AACCAAGU U UCAGGGGA	338	UCCCCUGA CUGAUGAG GCCGUUAGGC CGAA ACUUGGUU	1760
1757	ACCAAGUU U CAGGGGAC	339	GUCCCCUG CUGAUGAG GCCGUUAGGC CGAA AACUUGGU	1761
1758	CCAAGUUU C AGGGGACA	340	UGUCCCCU CUGAUGAG GCCGUUAGGC CGAA AAACUUGG	1762

1772	ACAUGAGU U UUCCAGCU	341	AGCUGGAA CUGAUGAG GCCGUUAGGC CGAA ACUCAUGU	1763
1773	CAUGAGUU U UCCAGCUU	342	AAGCUGGA CUGAUGAG GCCGUUAGGC CGAA AACUCAUG	1764
1774	AUGAGUUU U CCAGCUUU	343	AAAGCUGG CUGAUGAG GCCGUUAGGC CGAA AAACUCAU	1765
1775	UGAGUUUU C CAGCUUUU	344	AAAAGCUG CUGAUGAG GCCGUUAGGC CGAA AAAACUCA	1766
1781	UUCCAGCU U UUAUACAC	345	GUGUAUAA CUGAUGAG GCCGUUAGGC CGAA AGCUGGAA	1767
1782	UCCAGCUU U UAUACACA	346	UGUGUAUA CUGAUGAG GCCGUUAGGC CGAA AAGCUGGA	1768
1783	CCAGCUUU U AUACACAC	347	GUGUGUAU CUGAUGAG GCCGUUAGGC CGAA AAAGCUGG	1769
1784	CAGCUUUU A UACACACG	348	CGUGUGUA CUGAUGAG GCCGUUAGGC CGAA AAAAGCUG	1770
1786	GCUUUUAU A CACACGUA	349	UACGUGUG CUGAUGAG GCCGUUAGGC CGAA AUAAAAGC	1771
1794	ACACACGU A UCUCAUUU	350	AAAUGAGA CUGAUGAG GCCGUUAGGC CGAA ACGUGUGU	1772
1796	ACACGUAU C UCAUUUUU	351	AAAAAUGA CUGAUGAG GCCGUUAGGC CGAA AUACGUGU	1773
1798	ACGUAUCU C AUUUUUAU	352	AUAAAAAU CUGAUGAG GCCGUUAGGC CGAA AGAUACGU	1774
1801	UAUCUCAU U UUUAUCAA	353	UUGAUAAA CUGAUGAG GCCGUUAGGC CGAA AUGAGAUA	1775
1802	AUCUCAUU U UUAUCAAA	354	UUUGAUAA CUGAUGAG GCCGUUAGGC CGAA AAUGAGAU	1776
1803	UCUCAUUU U UAUCAAAA	355	UUUUGAUA CUGAUGAG GCCGUUAGGC CGAA AAAUGAGA	1777
1804	CUCAUUUU U AUCAAAAC	356	GUUUUGAU CUGAUGAG GCCGUUAGGC CGAA AAAAUGAG	1778
1805	UCAUUUUU A UCAAAACA	357	UGUUUUGA CUGAUGAG GCCGUUAGGC CGAA AAAAAUGA	1779
1807	AUUUUUAU C AAAACAUU	358	AAUGUUUU CUGAUGAG GCCGUUAGGC CGAA AUAAAAAU	1780

Input Sequence = AF016582 Cut Site = UH/.
Stem Length = 8 . Core Sequence = CUGAUGAG GCCGUUAGGC CGAA
AF016582 (Homo sapiens checkpoint kinase Chk1 (CHK1) mRNA; 1821 bp)

Underlined region can be any X sequence or linker as previously defined herein.

Table IV: Human Chk1 NCH Ribozyme and Substrate Sequence

Pos	Substrate	Seq ID	Ribozyme	Rz Seq ID
9	GGCCGGAC A GUCCGCCG	359	CGGCGGAC CUGAUGAG GCCGUUAGGC CGAA IUCCGGCC	1781
13	GGACAGUC C GCCGAGGU	360	ACCUCGGC CUGAUGAG GCCGUUAGGC CGAA IACUGUCC	1782
16	CAGUCCGC C GAGGUGCU	361	AGCACCUC CUGAUGAG GCCGUUAGGC CGAA ICGGACUG	1783
24	CGAGGUGC U CGGUGGAG	362	CUCCACCG CUGAUGAG GCCGUUAGGC CGAA ICACCUCG	1784
35	GUGGAGUC A UGGCAGUG	363	CACUGCCA CUGAUGAG GCCGUUAGGC CGAA IACUCCAC	1785
40	GUCAUGGC A GUGCCCUU	364	AAGGGCAC CUGAUGAG GCCGUUAGGC CGAA ICCAUGAC	1786
45	GGCAGUGC C CUUUGUGG	365	CCACAAAG CUGAUGAG GCCGUUAGGC CGAA ICACUGCC	1787
46	GCAGUGCC C UUUGUGGA	366	UCCACAAA CUGAUGAG GCCGUUAGGC CGAA IGCACUGC	1788
47	CAGUGCCC U UUGUGGAA	367	UUCCACAA CUGAUGAG GCCGUUAGGC CGAA IGGCACUG	1789
59	UGGAAGAC U GGGACUUG	368	CAAGUCCC CUGAUGAG GCCGUUAGGC CGAA IUCUUCCA	1790
65	ACUGGGAC U UGGUGCAA	369	UUGCACCA CUGAUGAG GCCGUUAGGC CGAA IUCCCAGU	1791
72	CUUGGUGC A AACCCUGG	370	CCAGGGUU CUGAUGAG GCCGUUAGGC CGAA ICACCAAG	1792
76	GUGCAAAC C CUGGGAGA	371	UCUCCCAG CUGAUGAG GCCGUUAGGC CGAA IUUUGCAC	1793
77	UGCAAACC C UGGGAGAA	372	UUCUCCCA CUGAUGAG GCCGUUAGGC CGAA IGUUUGCA	1794
78	GCAAACCC U GGGAGAAG	373	CUUCUCCC CUGAUGAG GCCGUUAGGC CGAA IGGUUUGC	1795
91	GAAGGUGC C UAUGGAGA	374	UCUCCAUA CUGAUGAG GCCGUUAGGC CGAA ICACCUUC	1796
92	AAGGUGCC U AUGGAGAA	375	UUCUCCAU CUGAUGAG GCCGUUAGGC CGAA IGCACCUU	1797
105	AGAAGUUC A ACUUGCUG	376	CAGCAAGU CUGAUGAG GCCGUUAGGC CGAA IAACUUCU	1798
108	AGUUCAAC U UGCUGUGA	377	UCACAGCA CUGAUGAG GCCGUUAGGC CGAA IUUGAACU	1799
112	CAACUUGC U GUGAAUAG	378	CUAUUCAC CUGAUGAG GCCGUUAGGC CGAA ICAAGUUG	1800
127	AGAGUAAC U GAAGAAGC	379	GCUUCUUC CUGAUGAG GCCGUUAGGC CGAA IUUACUCU	1801
136	GAAGAAGC A GUCGCAGU	380	ACUGCGAC CUGAUGAG GCCGUUAGGC CGAA ICUUCUUC	1802
142	GCAGUCGC A GUGAAGAU	381	AUCUUCAC CUGAUGAG GCCGUUAGGC CGAA ICGACUGC	1803
169	AAGCGUGC C GUAGACUG	382	CAGUCUAC CUGAUGAG GCCGUUAGGC CGAA ICACGCUU	1804
176	CCGUAGAC U GUCCAGAA	383	UUCUGGAC CUGAUGAG GCCGUUAGGC CGAA IUCUACGG	1805
180	AGACUGUC C AGAAAAUA	384	UAUUUUCU CUGAUGAG GCCGUUAGGC CGAA IACAGUCU	1806
181	GACUGUCC A GAAAAUAU	385	AUAUUUUC CUGAUGAG GCCGUUAGGC CGAA IGACAGUC	1807
203	AAGAGAUC U GUAUCAAU	386	AUUGAUAC CUGAUGAG GCCGUUAGGC CGAA IAUCUCUU	1808
209	UCUGUAUC A AUAAAAUG	387	CAUUUUAU CUGAUGAG GCCGUUAGGC CGAA IAUACAGA	1809
219	UAAAAUGC U AAAUCAUG	388	CAUGAUUU CUGAUGAG GCCGUUAGGC CGAA ICAUUUUA	1810
225	GCUAAAUC A UGAAAAUG	389	CAUUUUCA CUGAUGAG GCCGUUAGGC CGAA IAUUUAGC	1811
245	UAAAAUUC U AUGGUCAC	390	GUGACCAU CUGAUGAG GCCGUUAGGC CGAA IAAUUUUA	1812
252	CUAUGGUC A CAGGAGAG	391	CUCUCCUG CUGAUGAG GCCGUUAGGC CGAA IACCAUAG	1813
254	AUGGUCAC A GGAGAGAA	392	UUCUCUCC CUGAUGAG GCCGUUAGGC CGAA IUGACCAU	1814
266	GAGAAGGC A AUAUCCAA	393	UUGGAUAU CUGAUGAG GCCGUUAGGC CGAA ICCUUCUC	1815
272	GCAAUAUC C AAUAUUUA	394	UAAAUAUU CUGAUGAG GCCGUUAGGC CGAA IAUAUUGC	1816
273	CAAUAUCC A AUAUUUAU	395	AUAAAUAU CUGAUGAG GCCGUUAGGC CGAA IGAUAUUG	1817
285	UUUAUUUC U GGAGUACU	396	AGUACUCC CUGAUGAG GCCGUUAGGC CGAA IAAAUAAA	1818
293	UGGAGUAC U GUAGUGGA	397	UCCACUAC CUGAUGAG GCCGUUAGGC CGAA IUACUCCA	1819
309	AGGAGAGC U UUUUGACA	398	UGUCAAAA CUGAUGAG GCCGUUAGGC CGAA ICUCUCCU	1820
317	UUUUUGAC A GAAUAGAG	399	CUCUAUUC CUGAUGAG GCCGUUAGGC CGAA IUCAAAAA	1821
327	AAUAGAGC C AGACAUAG	400	CUAUGUCU CUGAUGAG GCCGUUAGGC CGAA ICUCUAUU	1822
328	AUAGAGCC A GACAUAGG	401	CCUAUGUC CUGAUGAG GCCGUUAGGC CGAA IGCUCUAU	1823
332	AGCCAGAC A UAGGCAUG	402	CAUGCCUA CUGAUGAG GCCGUUAGGC CGAA IUCUGGCU	1824
338	ACAUAGGC A UGCCUGAA	403	UUCAGGCA CUGAUGAG GCCGUUAGGC CGAA ICCUAUGU	1825

342	AGGCAUGC C UGAACCAG	404	CUGGUUCA CUGAUGAG GCCGUUAGGC CGAA ICAUGCCU	1826
343	GGCAUGCC U GAACCAGA	405	UCUGGUUC CUGAUGAG GCCGUUAGGC CGAA IGCAUGCC	1827
348	GCCUGAAC C AGAUGCUC	406	GAGCAUCU CUGAUGAG GCCGUUAGGC CGAA IUUCAGGC	1828
349	CCUGAACC A GAUGCUCA	407	UGAGCAUC CUGAUGAG GCCGUUAGGC CGAA IGUUCAGG	1829
355	CCAGAUGC U CAGAGAUU	408	AAUCUCUG CUGAUGAG GCCGUUAGGC CGAA ICAUCUGG	1830
357	AGAUGCUC A GAGAUUCU	409	AGAAUCUC CUGAUGAG GCCGUUAGGC CGAA IAGCAUCU	1831
365	AGAGAUUC U UCCAUCAA	410	UUGAUGGA CUGAUGAG GCCGUUAGGC CGAA IAAUCUCU	1832
368	GAUUCUUC C AUCAACUC	411	GAGUUGAU CUGAUGAG GCCGUUAGGC CGAA IAAGAAUC	1833
369	AUUCUUCC A UCAACUCA	412	UGAGUUGA CUGAUGAG GCCGUUAGGC CGAA IGAAGAAU	1834
372	CUUCCAUC A ACUCAUGG	413	CCAUGAGU CUGAUGAG GCCGUUAGGC CGAA IAUGGAAG	1835
375	CCAUCAAC U CAUGGCAG	414	CUGCCAUG CUGAUGAG GCCGUUAGGC CGAA IUUGAUGG	1836
377	AUCAACUC A UGGCAGGG	415	CCCUGCCA CUGAUGAG GCCGUUAGGC CGAA IAGUUGAU	1837
382	CUCAUGGC A GGGGUGGU	416	ACCACCCC CUGAUGAG GCCGUUAGGC CGAA ICCAUGAG	1838
396	GGUUUAUC U GCAUGGUA	417	UACCAUGC CUGAUGAG GCCGUUAGGC CGAA IAUAAACC	1839
399	UUAUCUGC A UGGUAUUG	418	CAAUACCA CUGAUGAG GCCGUUAGGC CGAA ICAGAUAA	1840
415	GGAAUAAC U CACAGGGA	419	UCCCUGUG CUGAUGAG GCCGUUAGGC CGAA IUUAUUCC	1841
417	AAUAACUC A CAGGGAUA	420	UAUCCCUG CUGAUGAG GCCGUUAGGC CGAA IAGUUAUU	1842
419	UAACUCAC A GGGAUAUU	421	AAUAUCCC CUGAUGAG GCCGUUAGGC CGAA IUGAGUUA	1843
432	UAUUAAAC C AGAAAAUC	422	GAUUUUCU CUGAUGAG GCCGUUAGGC CGAA IUUUAAUA	1844
433	AUUAAACC A GAAAAUCU	423	AGAUUUUC CUGAUGAG GCCGUUAGGC CGAA IGUUUAAU	1845
441	AGAAAAUC U UCUGUUGG	424	CCAACAGA CUGAUGAG GCCGUUAGGC CGAA IAUUUUCU	1846
444	AAAUCUUC U GUUGGAUG	425	CAUCCAAC CUGAUGAG GCCGUUAGGC CGAA IAAGAUUU	1847
464	GGGAUAAC C UCAAAAUC	426	GAUUUUGA CUGAUGAG GCCGUUAGGC CGAA IUUAUCCC	1848
465	GGAUAACC U CAAAAUCU	427	AGAUUUUG CUGAUGAG GCCGUUAGGC CGAA IGUUAUCC	1849
467	AUAACCUC A AAAUCUCA	428	UGAGAUUU CUGAUGAG GCCGUUAGGC CGAA IAGGUUAU	1850
473	UCAAAAUC U CAGACUUU	429	AAAGUCUG CUGAUGAG GCCGUUAGGC CGAA IAUUUUGA	1851
475	AAAAUCUC A GACUUUGG	430	CCAAAGUC CUGAUGAG GCCGUUAGGC CGAA IAGAUUUU	1852
479	UCUCAGAC U UUGGCUUG	431	CAAGCCAA CUGAUGAG GCCGUUAGGC CGAA IUCUGAGA	1853
485	ACUUUGGC U UGGCAACA	432	UGUUGCCA CUGAUGAG GCCGUUAGGC CGAA ICCAAAGU	1854
490	GGCUUGGC A ACAGUAUU	433	AAUACUGU CUGAUGAG GCCGUUAGGC CGAA ICCAAGCC	1855
493	UUGGCAAC A GUAUUUCG	434	CGAAAUAC CUGAUGAG GCCGUUAGGC CGAA IUUGCCAA	1856
530	UGUUGAAC A AGAUGUGU	435	ACACAUCU CUGAUGAG GCCGUUAGGC CGAA IUUCAACA	1857
544	UGUGGUAC U UUACCAUA	436	UAUGGUAA CUGAUGAG GCCGUUAGGC CGAA IUACCACA	1858
549	UACUUUAC C AUAUGUUG	437	CAACAUAU CUGAUGAG GCCGUUAGGC CGAA IUAAAGUA	1859
550	ACUUUACC A UAUGUUGC	438	GCAACAUA CUGAUGAG GCCGUUAGGC CGAA IGUAAAGU	1860
559	UAUGUUGC U CCAGAACU	439	AGUUCUGG CUGAUGAG GCCGUUAGGC CGAA ICAACAUA	1861
561	UGUUGCUC C AGAACUUC	440	GAAGUUCU CUGAUGAG GCCGUUAGGC CGAA IAGCAACA	1862
562	GUUGCUCC A GAACUUCU	441	AGAAGUUC CUGAUGAG GCCGUUAGGC CGAA IGAGCAAC	1863
567	UCCAGAAC U UCUGAAGA	442	UCUUCAGA CUGAUGAG GCCGUUAGGC CGAA IUUCUGGA	1864
570	AGAACUUC U GAAGAGAA	443	UUCUCUUC CUGAUGAG GCCGUUAGGC CGAA IAAGUUCU	1865
588	AGAAUUUC A UGCAGAAC	444	GUUCUGCA CUGAUGAG GCCGUUAGGC CGAA IAAAUUCU	1866
592	UUUCAUGC A GAACCAGU	445	ACUGGUUC CUGAUGAG GCCGUUAGGC CGAA ICAUGAAA	1867
597	UGCAGAAC C AGUUGAUG	446	CAUCAACU CUGAUGAG GCCGUUAGGC CGAA IUUCUGCA	1868
598	GCAGAACC A GUUGAUGU	447	ACAUCAAC CUGAUGAG GCCGUUAGGC CGAA IGUUCUGC	1869
613	GUUUGGUC C UGUGGAAU	448	AUUCCACA CUGAUGAG GCCGUUAGGC CGAA IACCAAAC	1870
614	UUUGGUCC U GUGGAAUA	449	UAUUCCAC CUGAUGAG GCCGUUAGGC CGAA IGACCAAA	1871
627	AAUAGUAC U UACUGCAA	450	UUGCAGUA CUGAUGAG GCCGUUAGGC CGAA IUACUAUU	1872
631	GUACUUAC U GCAAUGCU	451	AGCAUUGC CUGAUGAG GCCGUUAGGC CGAA IUAAGUAC	1873
634	CUUACUGC A AUGCUCGC	452	GCGAGCAU CUGAUGAG GCCGUUAGGC CGAA ICAGUAAG	1874

639	UGCAAUGC U CGCUGGAG	453	CUCCAGCG CUGAUGAG GCCGUUAGGC CGAA ICAUUGCA	1875
643	AUGCUCGC U GGAGAAUU	454	AAUUCUCC CUGAUGAG GCCGUUAGGC CGAA ICGAGCAU	1876
654	AGAAUUGC C AUGGGACC	455	GGUCCCAU CUGAUGAG GCCGUUAGGC CGAA ICAAUUCU	1877
655	GAAUUGCC A UGGGACCA	456	UGGUCCCA CUGAUGAG GCCGUUAGGC CGAA IGCAAUUC	1878
662	CAUGGGAC C AACCCAGU	457	ACUGGGUU CUGAUGAG GCCGUUAGGC CGAA IUCCCAUG	1879
663	AUGGGACC A ACCCAGUG	458	CACUGGGU CUGAUGAG GCCGUUAGGC CGAA IGUCCCAU	1880
666	GGACCAAC C CAGUGACA	459	UGUCACUG CUGAUGAG GCCGUUAGGC CGAA IUUGGUCC	1881
667	GACCAACC C AGUGACAG	460	CUGUCACU CUGAUGAG GCCGUUAGGC CGAA IGUUGGUC	1882
668	ACCAACCC A GUGACAGC	461	GCUGUCAC CUGAUGAG GCCGUUAGGC CGAA IGGUUGGU	1883
674	CCAGUGAC A GCUGUCAG	462	CUGACAGC CUGAUGAG GCCGUUAGGC CGAA IUCACUGG	1884
677	GUGACAGC U GUCAGGAG	463	CUCCUGAC CUGAUGAG GCCGUUAGGC CGAA ICUGUCAC	1885
681	CAGCUGUC A GGAGUAUU	464	AAUACUCC CUGAUGAG GCCGUUAGGC CGAA IACAGCUG	1886
691	GAGUAUUC U GACUGGAA	465	UUCCAGUC CUGAUGAG GCCGUUAGGC CGAA IAAUACUC	1887
695	AUUCUGAC U GGAAAGAA	466	UUCUUUCC CUGAUGAG GCCGUUAGGC CGAA IUCAGAAU	1888
712	AAAAAAAC A UACCUCAA	467	UUGAGGUA CUGAUGAG GCCGUUAGGC CGAA IUUUUUUU	1889
716	AAACAUAC C UCAACCCU	468	AGGGUUGA CUGAUGAG GCCGUUAGGC CGAA IUAUGUUU	1890
717	AACAUACC U CAACCCUU	469	AAGGGUUG CUGAUGAG GCCGUUAGGC CGAA IGUAUGUU	1891
719	CAUACCUC A ACCCUUGG	470	CCAAGGGU CUGAUGAG GCCGUUAGGC CGAA IAGGUAUG	1892
722	ACCUCAAC C CUUGGAAA	471	UUUCCAAG CUGAUGAG GCCGUUAGGC CGAA IUUGAGGU	1893
723	CCUCAACC C UUGGAAAA	472	UUUUCCAA CUGAUGAG GCCGUUAGGC CGAA IGUUGAGG	1894
724	CUCAACCC U UGGAAAAA	473	UUUUUCCA CUGAUGAG GCCGUUAGGC CGAA IGGUUGAG	1895
742	AUCGAUUC U GCUCCUCU	474	AGAGGAGC CUGAUGAG GCCGUUAGGC CGAA IAAUCGAU	1896
745	GAUUCUGC U CCUCUAGC	475	GCUAGAGG CUGAUGAG GCCGUUAGGC CGAA ICAGAAUC	1897
747	UUCUGCUC C UCUAGCUC	476	GAGCUAGA CUGAUGAG GCCGUUAGGC CGAA IAGCAGAA	1898
748	UCUGCUCC U CUAGCUCU	477	AGAGCUAG CUGAUGAG GCCGUUAGGC CGAA IGAGCAGA	1899
750	UGCUCCUC U AGCUCUGC	478	GCAGAGCU CUGAUGAG GCCGUUAGGC CGAA IAGGAGCA	1900
754	CCUCUAGC U CUGCUGCA	479	UGCAGCAG CUGAUGAG GCCGUUAGGC CGAA ICUAGAGG	1901
756	UCUAGCUC U GCUGCAUA	480	UAUGCAGC CUGAUGAG GCCGUUAGGC CGAA IAGCUAGA	1902
759	AGCUCUGC U GCAUAAAA	481	UUUUAUGC CUGAUGAG GCCGUUAGGC CGAA ICAGAGCU	1903
762	UCUGCUGC A UAAAAUCU	482	AGAUUUUA CUGAUGAG GCCGUUAGGC CGAA ICAGCAGA	1904
770	AUAAAAUC U UAGUUGAG	483	CUCAACUA CUGAUGAG GCCGUUAGGC CGAA IAUUUUAU	1905
783	UGAGAAUC C AUCAGCAA	484	UUGCUGAU CUGAUGAG GCCGUUAGGC CGAA IAUUCUCA	1906
784	GAGAAUCC A UCAGCAAG	485	CUUGCUGA CUGAUGAG GCCGUUAGGC CGAA IGAUUCUC	1907
787	AAUCCAUC A GCAAGAAU	486	AUUCUUGC CUGAUGAG GCCGUUAGGC CGAA IAUGGAUU	1908
790	CCAUCAGC A AGAAUUAC	487	GUAAUUCU CUGAUGAG GCCGUUAGGC CGAA ICUGAUGG	1909
799	AGAAUUAC C AUUCCAGA	488	UCUGGAAU CUGAUGAG GCCGUUAGGC CGAA IUAAUUCU	1910
800	GAAUUACC A UUCCAGAC	489	GUCUGGAA CUGAUGAG GCCGUUAGGC CGAA IGUAAUUC	1911
804	UACCAUUC C AGACAUCA	490	UGAUGUCU CUGAUGAG GCCGUUAGGC CGAA IAAUGGUA	1912
805	ACCAUUCC A GACAUCAA	491	UUGAUGUC CUGAUGAG GCCGUUAGGC CGAA IGAAUGGU	1913
809	UUCCAGAC A UCAAAAAA	492	UUUUUUGA CUGAUGAG GCCGUUAGGC CGAA IUCUGGAA	1914
812	CAGACAUC A AAAAAGAU	493	AUCUUUUU CUGAUGAG GCCGUUAGGC CGAA IAUGUCUG	1915
830	GAUGGUAC A ACAAACCC	494	GGGUUUGU CUGAUGAG GCCGUUAGGC CGAA IUACCAUC	1916
833	GGUACAAC A AACCCCUC	495	GAGGGGUU CUGAUGAG GCCGUUAGGC CGAA IUUGUACC	1917
837	CAACAAAC C CCUCAAGA	496	UCUUGAGG CUGAUGAG GCCGUUAGGC CGAA IUUUGUUG	1918
838	AACAAACC C CUCAAGAA	497	UUCUUGAG CUGAUGAG GCCGUUAGGC CGAA IGUUUGUU	1919
839	ACAAACCC C UCAAGAAA	498	UUUCUUGA CUGAUGAG GCCGUUAGGC CGAA IGGUUUGU	1920
840	CAAACCCC U CAAGAAAG	499	CUUUCUUG CUGAUGAG GCCGUUAGGC CGAA IGGGUUUG	1921
842	AACCCCUC A AGAAAGGG	500	CCCUUUCU CUGAUGAG GCCGUUAGGC CGAA IAGGGGUU	1922
853	AAAGGGGC A AAAAGGCC	501	GGCCUUUU CUGAUGAG GCCGUUAGGC CGAA ICCCCUUU	1923

861	AAAAAGGC C CCGAGUCA	502	UGACUCGG CUGAUGAG GCCGUUAGGC CGAA ICCUUUUU	1924
862	AAAAGGCC C CGAGUCAC	503	GUGACUCG CUGAUGAG GCCGUUAGGC CGAA IGCCUUUU	1925
863	AAAGGCCC C GAGUCACU	504	AGUGACUC CUGAUGAG GCCGUUAGGC CGAA IGGCCUUU	1926
869	CCCGAGUC A CUUCAGGU	505	ACCUGAAG CUGAUGAG GCCGUUAGGC CGAA IACUCGGG	1927
871	CGAGUCAC U UCAGGUGG	506	CCACCUGA CUGAUGAG GCCGUUAGGC CGAA IUGACUCG	1928
874	GUCACUUC A GGUGGUGU	507	ACACCACC CUGAUGAG GCCGUUAGGC CGAA IAAGUGAC	1929
886	GGUGUGUC A GAGUCUCC	508	GGAGACUC CUGAUGAG GCCGUUAGGC CGAA IACACACC	1930
892	UCAGAGUC U CCCAGUGG	509	CCACUGGG CUGAUGAG GCCGUUAGGC CGAA IACUCUGA	1931
894	AGAGUCUC C CAGUGGAU	510	AUCCACUG CUGAUGAG GCCGUUAGGC CGAA IAGACUCU	1932
895	GAGUCUCC C AGUGGAUU	511	AAUCCACU CUGAUGAG GCCGUUAGGC CGAA IGAGACUC	1933
896	AGUCUCCC A GUGGAUUU	512	AAAUCCAC CUGAUGAG GCCGUUAGGC CGAA IGGAGACU	1934
907	GGAUUUUC U AAGCACAU	513	AUGUGCUU CUGAUGAG GCCGUUAGGC CGAA IAAAAUCC	1935
912	UUCUAAGC A CAUUCAAU	514	AUUGAAUG CUGAUGAG GCCGUUAGGC CGAA ICUUAGAA	1936
914	CUAAGCAC A UUCAAUCC	515	GGAUUGAA CUGAUGAG GCCGUUAGGC CGAA IUGCUUAG	1937
918	GCACAUUC A AUCCAAUU	516	AAUUGGAU CUGAUGAG GCCGUUAGGC CGAA IAAUGUGC	1938
922	AUUCAAUC C AAUUUGGA	517	UCCAAAUU CUGAUGAG GCCGUUAGGC CGAA IAUUGAAU	1939
923	UUCAAUCC A AUUUGGAC	518	GUCCAAAU CUGAUGAG GCCGUUAGGC CGAA IGAUUGAA	1940
932	AUUUGGAC U UCUCUCCA	519	UGGAGAGA CUGAUGAG GCCGUUAGGC CGAA IUCCAAAU	1941
935	UGGACUUC U CUCCAGUA	520	UACUGGAG CUGAUGAG GCCGUUAGGC CGAA IAAGUCCA	1942
937	GACUUCUC U CCAGUAAA	521	UUUACUGG CUGAUGAG GCCGUUAGGC CGAA IAGAAGUC	1943
939	CUUCUCUC C AGUAAACA	522	UGUUUACU CUGAUGAG GCCGUUAGGC CGAA IAGAGAAG	1944
940	UUCUCUCC A GUAAACAG	523	CUGUUUAC CUGAUGAG GCCGUUAGGC CGAA IGAGAGAA	1945
947	CAGUAAAC A GUGCUUCU	524	AGAAGCAC CUGAUGAG GCCGUUAGGC CGAA IUUUACUG	1946
952	AACAGUGC U UCUAGUGA	525	UCACUAGA CUGAUGAG GCCGUUAGGC CGAA ICACUGUU	1947
955	AGUGCUUC U AGUGAAGA	526	UCUUCACU CUGAUGAG GCCGUUAGGC CGAA IAAGCACU	1948
977	UGAAGUAC U CCAGUUCU	527	AGAACUGG CUGAUGAG GCCGUUAGGC CGAA IUACUUCA	1949
979	AAGUACUC C AGUUCUCA	528	UGAGAACU CUGAUGAG GCCGUUAGGC CGAA IAGUACUU	1950
980	AGUACUCC A GUUCUCAG	529	CUGAGAAC CUGAUGAG GCCGUUAGGC CGAA IGAGUACU	1951
985	UCCAGUUC U CAGCCAGA	530	UCUGGCUG CUGAUGAG GCCGUUAGGC CGAA IAACUGGA	1952
987	CAGUUCUC A GCCAGAAC	531	GUUCUGGC CUGAUGAG GCCGUUAGGC CGAA IAGAACUG	1953
990	UUCUCAGC C AGAACCCC	532	GGGGUUCU CUGAUGAG GCCGUUAGGC CGAA ICUGAGAA	1954
991	UCUCAGCC A GAACCCCG	533	CGGGGUUC CUGAUGAG GCCGUUAGGC CGAA IGCUGAGA	1955
996	GCCAGAAC C CCGCACAG	534	CUGUGCGG CUGAUGAG GCCGUUAGGC CGAA IUUCUGGC	1956
997	CCAGAACC C CGCACAGG	535	CCUGUGCG CUGAUGAG GCCGUUAGGC CGAA IGUUCUGG	1957
998	CAGAACCC C GCACAGGU	536	ACCUGUGC CUGAUGAG GCCGUUAGGC CGAA IGGUUCUG	1958
1001	AACCCCGC A CAGGUCUU	537	AAGACCUG CUGAUGAG GCCGUUAGGC CGAA ICGGGGUU	1959
1003	CCCCGCAC A GGUCUUUC	538	GAAAGACC CUGAUGAG GCCGUUAGGC CGAA IUGCGGGG	1960
1008	CACAGGUC U UUCCUUAU	539	AUAAGGAA CUGAUGAG GCCGUUAGGC CGAA IACCUGUG	1961
1012	GGUCUUUC C UUAUGGGA	540	UCCCAUAA CUGAUGAG GCCGUUAGGC CGAA IAAAGACC	1962
1013	GUCUUUCC U UAUGGGAU	541	AUCCCAUA CUGAUGAG GCCGUUAGGC CGAA IGAAAGAC	1963
1024	UGGGAUAC C AGCCCCUC	542	GAGGGGCU CUGAUGAG GCCGUUAGGC CGAA IUAUCCCA	1964
1025	GGGAUACC A GCCCCUCA	543	UGAGGGGC CUGAUGAG GCCGUUAGGC CGAA IGUAUCCC	1965
1028	AUACCAGC C CCUCAUAC	544	GUAUGAGG CUGAUGAG GCCGUUAGGC CGAA ICUGGUAU	1966
1029	UACCAGCC C CUCAUACA	545	UGUAUGAG CUGAUGAG GCCGUUAGGC CGAA IGCUGGUA	1967
1030	ACCAGCCC C UCAUACAU	546	AUGUAUGA CUGAUGAG GCCGUUAGGC CGAA IGGCUGGU	1968
1031	CCAGCCCC U CAUACAUU	547	AAUGUAUG CUGAUGAG GCCGUUAGGC CGAA IGGGCUGG	1969
1033	AGCCCCUC A UACAUUGA	548	UCAAUGUA CUGAUGAG GCCGUUAGGC CGAA IAGGGGCU	1970
1037	CCUCAUAC A UUGAUAAA	549	UUUAUCAA CUGAUGAG GCCGUUAGGC CGAA IUAUGAGG	1971
1053	AUUGGUAC A AGGGAUCA	550	UGAUCCCU CUGAUGAG GCCGUUAGGC CGAA IUACCAAU	1972

1061	AAGGGAUC A GCUUUUCC	551	GGAAAAGC CUGAUGAG GCCGUUAGGC CGAA IAUCCCUU	1973
1064	GGAUCAGC U UUUCCCAG	552	CUGGGAAA CUGAUGAG GCCGUUAGGC CGAA ICUGAUCC	1974
1069	AGCUUUUC C CAGCCCAC	553	GUGGGCUG CUGAUGAG GCCGUUAGGC CGAA IAAAAGCU	1975
1070	GCUUUUCC C AGCCCACA	554	UGUGGGCU CUGAUGAG GCCGUUAGGC CGAA IGAAAAGC	1976
1071	CUUUUCCC A GCCCACAU	555	AUGUGGGC CUGAUGAG GCCGUUAGGC CGAA IGGAAAAG	1977
1074	UUCCCAGC C CACAUGUC	556	GACAUGUG CUGAUGAG GCCGUUAGGC CGAA ICUGGGAA	1978
1075	UCCCAGCC C ACAUGUCC	557	GGACAUGU CUGAUGAG GCCGUUAGGC CGAA IGCUGGGA	1979
1076	CCCAGCCC A CAUGUCCU	558	AGGACAUG CUGAUGAG GCCGUUAGGC CGAA IGGCUGGG	1980
1078	CAGCCCAC A UGUCCUGA	559	UCAGGACA CUGAUGAG GCCGUUAGGC CGAA IUGGGCUG	1981
1083	CACAUGUC C UGAUCAUA	560	UAUGAUCA CUGAUGAG GCCGUUAGGC CGAA IACAUGUG	1982
1084	ACAUGUCC U GAUCAUAU	561	AUAUGAUC CUGAUGAG GCCGUUAGGC CGAA IGACAUGU	1983
1089	UCCUGAUC A UAUGCUUU	562	AAAGCAUA CUGAUGAG GCCGUUAGGC CGAA IAUCAGGA	1984
1095	UCAUAUGC U UUUGAAUA	563	UAUUCAAA CUGAUGAG GCCGUUAGGC CGAA ICAUAUGA	1985
1107	GAAUAGUC A GUUACUUG	564	CAAGUAAC CUGAUGAG GCCGUUAGGC CGAA IACUAUUC	1986
1113	UCAGUUAC U UGGCACCC	565	GGGUGCCA CUGAUGAG GCCGUUAGGC CGAA IUAACUGA	1987
1118	UACUUGGC A CCCCAGGA	566	UCCUGGGG CUGAUGAG GCCGUUAGGC CGAA ICCAAGUA	1988
1120	CUUGGCAC C CCAGGAUC	567	GAUCCUGG CUGAUGAG GCCGUUAGGC CGAA IUGCCAAG	1989
1121	UUGGCACC C CAGGAUCC	568	GGAUCCUG CUGAUGAG GCCGUUAGGC CGAA IGUGCCAA	1990
1122	UGGCACCC C AGGAUCCU	569	AGGAUCCU CUGAUGAG GCCGUUAGGC CGAA IGGUGCCA	1991
1123	GGCACCCC A GGAUCCUC	570	GAGGAUCC CUGAUGAG GCCGUUAGGC CGAA IGGGUGCC	1992
1129	CCAGGAUC C UCACAGAA	571	UUCUGUGA CUGAUGAG GCCGUUAGGC CGAA IAUCCUGG	1993
1130	CAGGAUCC U CACAGAAC	572	GUUCUGUG CUGAUGAG GCCGUUAGGC CGAA IGAUCCUG	1994
1132	GGAUCCUC A CAGAACCC	573	GGGUUCUG CUGAUGAG GCCGUUAGGC CGAA IAGGAUCC	1995
1134	AUCCUCAC A GAACCCCU	574	AGGGGUUC CUGAUGAG GCCGUUAGGC CGAA IUGAGGAU	1996
1139	CACAGAAC C CCUGGCAG	575	CUGCCAGG CUGAUGAG GCCGUUAGGC CGAA IUUCUGUG	1997
1140	ACAGAACC C CUGGCAGC	576	GCUGCCAG CUGAUGAG GCCGUUAGGC CGAA IGUUCUGU	1998
1141	CAGAACCC C UGGCAGCG	577	CGCUGCCA CUGAUGAG GCCGUUAGGC CGAA IGGUUCUG	1999
1142	AGAACCCC U GGCAGCGG	578	CCGCUGCC CUGAUGAG GCCGUUAGGC CGAA IGGGUUCU	2000
1146	CCCCUGGC A GCGGUUGG	579	CCAACCGC CUGAUGAG GCCGUUAGGC CGAA ICCAGGGG	2001
1157	GGUUGGUC A AAAGAAUG	580	CAUUCUUU CUGAUGAG GCCGUUAGGC CGAA IACCAACC	2002
1168	AGAAUGAC A CGAUUCUU	581	AAGAAUCG CUGAUGAG GCCGUUAGGC CGAA IUCAUUCU	2003
1175	CACGAUUC U UUACCAAA	582	UUUGGUAA CUGAUGAG GCCGUUAGGC CGAA IAAUCGUG	2004
1180	UUCUUUAC C AAAUUGGA	583	UCCAAUUU CUGAUGAG GCCGUUAGGC CGAA IUAAAGAA	2005
1181	UCUUUACC A AAUUGGAU	584	AUCCAAUU CUGAUGAG GCCGUUAGGC CGAA IGUAAAGA	2006
1192	UUGGAUGC A GACAAAUC	585	GAUUUGUC CUGAUGAG GCCGUUAGGC CGAA ICAUCCAA	2007
1196	AUGCAGAC A AAUCUUAU	586	AUAAGAUU CUGAUGAG GCCGUUAGGC CGAA IUCUGCAU	2008
1201	GACAAAUC U UAUCAAUG	587	CAUUGAUA CUGAUGAG GCCGUUAGGC CGAA IAUUUGUC	2009
1206	AUCUUAUC A AUGCCUGA	588	UCAGGCAU CUGAUGAG GCCGUUAGGC CGAA IAUAAGAU	2010
1211	AUCAAUGC C UGAAAGAG	589	CUCUUUCA CUGAUGAG GCCGUUAGGC CGAA ICAUUGAU	2011
1212	UCAAUGCC U GAAAGAGA	590	UCUCUUUC CUGAUGAG GCCGUUAGGC CGAA IGCAUUGA	2012
1222	AAAGAGAC U UGUGAGAA	591	UUCUCACA CUGAUGAG GCCGUUAGGC CGAA IUCUCUUU	2013
1238	AGUUGGGC U AUCAAUGG	592	CCAUUGAU CUGAUGAG GCCGUUAGGC CGAA ICCCAACU	2014
1242	GGGCUAUC A AUGGAAGA	593	UCUUCCAU CUGAUGAG GCCGUUAGGC CGAA IAUAGCCC	2015
1266	UAUGAAUC A GGUUACUA	594	UAGUAACC CUGAUGAG GCCGUUAGGC CGAA IAUUCAUA	2016
1273	CAGGUUAC U AUAUCAAC	595	GUUGAUAU CUGAUGAG GCCGUUAGGC CGAA IUAACCUG	2017
1279	ACUAUAUC A ACAACUGA	596	UCAGUUGU CUGAUGAG GCCGUUAGGC CGAA IAUAUAGU	2018
1282	AUAUCAAC A ACUGAUAG	597	CUAUCAGU CUGAUGAG GCCGUUAGGC CGAA IUUGAUAU	2019
1285	UCAACAAC U GAUAGGAG	598	CUCCUAUC CUGAUGAG GCCGUUAGGC CGAA IUUGUUGA	2020
1298	GGAGAAAC A AUAAACUC	599	GAGUUUAU CUGAUGAG GCCGUUAGGC CGAA IUUUCUCC	2021

1305	CAAUAAAC U CAUUUUCA	600	UGAAAAUG CUGAUGAG GCCGUUAGGC CGAA IUUUAUUG	2022
1307	AUAAACUC A UUUUCAAA	601	UUUGAAAA CUGAUGAG GCCGUUAGGC CGAA IAGUUUAU	2023
1313	UCAUUUUC A AAGUGAAU	602	AUUCACUU CUGAUGAG GCCGUUAGGC CGAA IAAAAUGA	2024
1355	UGGUUGAC U UCCGGCUU	603	AAGCCGGA CUGAUGAG GCCGUUAGGC CGAA IUCAACCA	2025
1358	UUGACUUC C GGCUUUCU	604	AGAAAGCC CUGAUGAG GCCGUUAGGC CGAA IAAGUCAA	2026
1362	CUUCCGGC U UUCUAAGG	605	CCUUAGAA CUGAUGAG GCCGUUAGGC CGAA ICCGGAAG	2027
1366	CGGCUUUC U AAGGGUGA	606	UCACCCUU CUGAUGAG GCCGUUAGGC CGAA IAAAGCCG	2028
1388	UGGAGUUC A AGAGACAC	607	GUGUCUCU CUGAUGAG GCCGUUAGGC CGAA IAACUCCA	2029
1395	CAAGAGAC A CUUCCUGA	608	UCAGGAAG CUGAUGAG GCCGUUAGGC CGAA IUCUCUUG	2030
1397	AGAGACAC U UCCUGAAG	609	CUUCAGGA CUGAUGAG GCCGUUAGGC CGAA IUGUCUCU	2031
1400	GACACUUC C UGAAGAUU	610	AAUCUUCA CUGAUGAG GCCGUUAGGC CGAA IAAGUGUC	2032
1401	ACACUUCC U GAAGAUUA	611	UAAUCUUC CUGAUGAG GCCGUUAGGC CGAA IGAAGUGU	2033
1419	AGGGAAGC U GAUUGAUA	612	UAUCAAUC CUGAUGAG GCCGUUAGGC CGAA ICUUCCCU	2034
1436	UUGUGAGC A GCCAGAAG	613	CUUCUGGC CUGAUGAG GCCGUUAGGC CGAA ICUCACAA	2035
1439	UGAGCAGC C AGAAGGUU	614	AACCUUCU CUGAUGAG GCCGUUAGGC CGAA ICUGCUCA	2036
1440	GAGCAGCC A GAAGGUUU	615	AAACCUUC CUGAUGAG GCCGUUAGGC CGAA IGCUGCUC	2037
1452	GGUUUGGC U UCCUGCCA	616	UGGCAGGA CUGAUGAG GCCGUUAGGC CGAA ICCAAACC	2038
1455	UUGGCUUC C UGCCACAU	617	AUGUGGCA CUGAUGAG GCCGUUAGGC CGAA IAAGCCAA	2039
1456	UGGCUUCC U GCCACAUG	618	CAUGUGGC CUGAUGAG GCCGUUAGGC CGAA IGAAGCCA	2040
1459	CUUCCUGC C ACAUGAUC	619	GAUCAUGU CUGAUGAG GCCGUUAGGC CGAA ICAGGAAG	2041
1460	UUCCUGCC A CAUGAUCG	620	CGAUCAUG CUGAUGAG GCCGUUAGGC CGAA IGCAGGAA	2042
1462	CCUGCCAC A UGAUCGGA	621	UCCGAUCA CUGAUGAG GCCGUUAGGC CGAA IUGGCAGG	2043
1472	GAUCGGAC C AUCGGCUC	622	GAGCCGAU CUGAUGAG GCCGUUAGGC CGAA IUCCGAUC	2044
1473	AUCGGACC A UCGGCUCU	623	AGAGCCGA CUGAUGAG GCCGUUAGGC CGAA IGUCCGAU	2045
1479	CCAUCGGC U CUGGGGAA	624	UUCCCCAG CUGAUGAG GCCGUUAGGC CGAA ICCGAUGG	2046
1481	AUCGGCUC U GGGGAAUC	625	GAUUCCCC CUGAUGAG GCCGUUAGGC CGAA IAGCCGAU	2047
1490	GGGGAAUC C UGGUGAAU	626	AUUCACCA CUGAUGAG GCCGUUAGGC CGAA IAUUCCCC	2048
1491	GGGAAUCC U GGUGAAUA	627	UAUUCACC CUGAUGAG GCCGUUAGGC CGAA IGAUUCCC	2049
1506	UAUAGUGC U GCUAUGUU	628	AACAUAGC CUGAUGAG GCCGUUAGGC CGAA ICACUAUA	2050
1509	AGUGCUGC U AUGUUGAC	629	GUCAACAU CUGAUGAG GCCGUUAGGC CGAA ICAGCACU	2051
1518	AUGUUGAC A UUAUUCUU	630	AAGAAUAA CUGAUGAG GCCGUUAGGC CGAA IUCAACAU	2052
1525	CAUUAUUC U UCCUAGAG	631	CUCUAGGA CUGAUGAG GCCGUUAGGC CGAA IAAUAAUG	2053
1528	UAUUCUUC C UAGAGAAG	632	CUUCUCUA CUGAUGAG GCCGUUAGGC CGAA IAAGAAUA	2054
1529	AUUCUUCC U AGAGAAGA	633	UCUUCUCU CUGAUGAG GCCGUUAGGC CGAA IGAAGAAU	2055
1543	AGAUUAUC C UGUCCUGC	634	GCAGGACA CUGAUGAG GCCGUUAGGC CGAA IAUAAUCU	2056
1544	GAUUAUCC U GUCCUGCA	635	UGCAGGAC CUGAUGAG GCCGUUAGGC CGAA IGAUAAUC	2057
1548	AUCCUGUC C UGCAAACU	636	AGUUUGCA CUGAUGAG GCCGUUAGGC CGAA IACAGGAU	2058
1549	UCCUGUCC U GCAAACUG	637	CAGUUUGC CUGAUGAG GCCGUUAGGC CGAA IGACAGGA	2059
1552	UGUCCUGC A AACUGCAA	638	UUGCAGUU CUGAUGAG GCCGUUAGGC CGAA ICAGGACA	2060
1556	CUGCAAAC U GCAAAUAG	639	CUAUUUGC CUGAUGAG GCCGUUAGGC CGAA IUUUGCAG	2061
1559	CAAACUGC A AAUAGUAG	640	CUACUAUU CUGAUGAG GCCGUUAGGC CGAA ICAGUUUG	2062
1571	AGUAGUUC C UGAAGUGU	641	ACACUUCA CUGAUGAG GCCGUUAGGC CGAA IAACUACU	2063
1572	GUAGUUCC U GAAGUGUU	642	AACACUUC CUGAUGAG GCCGUUAGGC CGAA IGAACUAC	2064
1582	AAGUGUUC A CUUCCCUG	643	CAGGGAAG CUGAUGAG GCCGUUAGGC CGAA IAACACUU	2065
1584	GUGUUCAC U UCCCUGUU	644	AACAGGGA CUGAUGAG GCCGUUAGGC CGAA IUGAACAC	2066
1587	UUCACUUC C CUGUUUAU	645	AUAAACAG CUGAUGAG GCCGUUAGGC CGAA IAAGUGAA	2067
1588	UCACUUCC C UGUUUAUC	646	GAUAAACA CUGAUGAG GCCGUUAGGC CGAA IGAAGUGA	2068
1589	CACUUCCC U GUUUAUCC	647	GGAUAAAC CUGAUGAG GCCGUUAGGC CGAA IGGAAGUG	2069
1597	UGUUUAUC C AAACAUCU	648	AGAUGUUU CUGAUGAG GCCGUUAGGC CGAA IAUAAACA	2070

1598	GUUUAUCC A AACAUCUU	649	AAGAUGUU CUGAUGAG GCCGUUAGGC CGAA IGAUAAAC	2071
1602	AUCCAAAC A UCUUCCAA	650	UUGGAAGA CUGAUGAG GCCGUUAGGC CGAA IUUUGGAU	2072
1605	CAAACAUC U UCCAAUUU	651	AAAUUGGA CUGAUGAG GCCGUUAGGC CGAA IAUGUUUG	2073
1608	ACAUCUUC C AAUUUAUU	652	AAUAAAUU CUGAUGAG GCCGUUAGGC CGAA IAAGAUGU	2074
1609	CAUCUUCC A AUUUAUUU	653	AAAUAAAU CUGAUGAG GCCGUUAGGC CGAA IGAAGAUG	2075
1630	UGUUCGGC A UACAAAUA	654	UAUUUGUA CUGAUGAG GCCGUUAGGC CGAA ICCGAACA	2076
1634	CGGCAUAC A AAUAAUAC	655	GUAUUAUU CUGAUGAG GCCGUUAGGC CGAA IUAUGCCG	2077
1643	AAUAAUAC C UAUAUCUU	656	AAGAUAUA CUGAUGAG GCCGUUAGGC CGAA IUAUUAUU	2078
1644	AUAAUACC U AUAUCUUA	657	UAAGAUAU CUGAUGAG GCCGUUAGGC CGAA IGUAUUAU	2079
1650	CCUAUAUC U UAAUUGUA	658	UACAAUUA CUGAUGAG GCCGUUAGGC CGAA IAUAUAGG	2080
1662	UUGUAAGC A AAACUUUG	659	CAAAGUUU CUGAUGAG GCCGUUAGGC CGAA ICUUACAA	2081
1667	AGCAAAAC U UUGGGGAA	660	UUCCCCAA CUGAUGAG GCCGUUAGGC CGAA IUUUUGCU	2082
1692	UAGAAUUC A UUUGAUUA	661	UAAUCAAA CUGAUGAG GCCGUUAGGC CGAA IAAUUCUA	2083
1705	AUUAUUUC U UCAUGUGU	662	ACACAUGA CUGAUGAG GCCGUUAGGC CGAA IAAAUAAU	2084
1708	AUUUCUUC A UGUGUGUU	663	AACACACA CUGAUGAG GCCGUUAGGC CGAA IAAGAAAU	2085
1724	UUAGUAUC U GAAUUUGA	664	UCAAAUUC CUGAUGAG GCCGUUAGGC CGAA IAUACUAA	2086
1736	UUUGAAAC U CAUCUGGU	665	ACCAGAUG CUGAUGAG GCCGUUAGGC CGAA IUUUCAAA	2087
1738	UGAAACUC A UCUGGUGG	666	CCACCAGA CUGAUGAG GCCGUUAGGC CGAA IAGUUUCA	2088
1741	AACUCAUC U GGUGGAAA	667	UUUCCACC CUGAUGAG GCCGUUAGGC CGAA IAUGAGUU	2089
1751	GUGGAAAC C AAGUUUCA	668	UGAAACUU CUGAUGAG GCCGUUAGGC CGAA IUUUCCAC	2090
1752	UGGAAACC A AGUUUCAG	669	CUGAAACU CUGAUGAG GCCGUUAGGC CGAA IGUUUCCA	2091
1759	CAAGUUUC A GGGGACAU	670	AUGUCCCC CUGAUGAG GCCGUUAGGC CGAA IAAACUUG	2092
1766	CAGGGGAC A UGAGUUUU	671	AAAACUCA CUGAUGAG GCCGUUAGGC CGAA IUCCCCUG	2093
1776	GAGUUUUC C AGCUUUUA	672	UAAAAGCU CUGAUGAG GCCGUUAGGC CGAA IAAAACUC	2094
1777	AGUUUUCC A GCUUUUAU	673	AUAAAAGC CUGAUGAG GCCGUUAGGC CGAA IGAAAACU	2095
1780	UUUCCAGC U UUUAUACA	674	UGUAUAAA CUGAUGAG GCCGUUAGGC CGAA ICUGGAAA	2096
1788	UUUUAUAC A CACGUAUC	675	GAUACGUG CUGAUGAG GCCGUUAGGC CGAA IUAUAAAA	2097
1790	UUAUACAC A CGUAUCUC	676	GAGAUACG CUGAUGAG GCCGUUAGGC CGAA IUGUAUAA	2098
1797	CACGUAUC U CAUUUUUA	677	UAAAAAUG CUGAUGAG GCCGUUAGGC CGAA IAUACGUG	2099
1799	CGUAUCUC A UUUUUAUC	678	GAUAAAAA CUGAUGAG GCCGUUAGGC CGAA IAGAUACG	2100
1808	UUUUUAUC A AAACAUUU	679	AAAUGUUU CUGAUGAG GCCGUUAGGC CGAA IAUAAAAA	2101
1813	AUCAAAAC A UUUUGUUU	680	AAACAAAA CUGAUGAG GCCGUUAGGC CGAA IUUUUGAU	2102

Input Sequence = AF016582 Cut Site = CH/.
Stem Length = 8 . Core Sequence = CUGAUGAG GCCGUUAGGC CGAA
AF016582 (Homo sapiens checkpoint kinase Chk1 (CHK1) mRNA; 1821 bp)

Underlined region can be any X sequence or linker as previously defined herein.
I = Inosine

Table V: Human Chk1 G-Cleaver Ribozyme and Substrate Sequence

Pos	Substrate	Seq ID	Ribozyme	Rz Seq ID
14	GACAGUCC G CCGAGGUG	681	CACCUCGG UGAUGGCAUGCACUAUGCGCG GGACUGUC	2103
17	AGUCCGCC G AGGUGCUC	682	GAGCACCU UGAUGGCAUGCACUAUGCGCG GGCGGACU	2104
22	GCCGAGGU G CUCGGUGG	683	CCACCGAG UGAUGGCAUGCACUAUGCGCG ACCUCGGC	2105
43	AUGGCAGU G CCCUUUGU	684	ACAAAGGG UGAUGGCAUGCACUAUGCGCG ACUGCCAU	2106
50	UGCCCUUU G UGGAAGAC	685	GUCUUCCA UGAUGGCAUGCACUAUGCGCG AAAGGGCA	2107
70	GACUUGGU G CAAACCCU	686	AGGGUUUG UGAUGGCAUGCACUAUGCGCG ACCAAGUC	2108
89	GAGAAGGU G CCUAUGGA	687	UCCAUAGG UGAUGGCAUGCACUAUGCGCG ACCUUCUC	2109
110	UUCAACUU G CUGUGAAU	688	AUUCACAG UGAUGGCAUGCACUAUGCGCG AAGUUGAA	2110
113	AACUUGCU G UGAAUAGA	689	UCUAUUCA UGAUGGCAUGCACUAUGCGCG AGCAAGUU	2111
115	CUUGCUGU G AAUAGAGU	690	ACUCUAUU UGAUGGCAUGCACUAUGCGCG ACAGCAAG	2112
128	GAGUAACU G AAGAAGCA	691	UGCUUCUU UGAUGGCAUGCACUAUGCGCG AGUUACUC	2113
140	AAGCAGUC G CAGUGAAG	692	CUUCACUG UGAUGGCAUGCACUAUGCGCG GACUGCUU	2114
145	GUCGCAGU G AAGAUUGU	693	ACAAUCUU UGAUGGCAUGCACUAUGCGCG ACUGCGAC	2115
152	UGAAGAUU G UAGAUAUG	694	CAUAUCUA UGAUGGCAUGCACUAUGCGCG AAUCUUCA	2116
160	GUAGAUAU G AAGCGUGC	695	GCACGCUU UGAUGGCAUGCACUAUGCGCG AUAUCUAC	2117
167	UGAAGCGU G CCGUAGAC	696	GUCUACGG UGAUGGCAUGCACUAUGCGCG ACGCUUCA	2118
177	CGUAGACU G UCCAGAAA	697	UUUCUGGA UGAUGGCAUGCACUAUGCGCG AGUCUACG	2119
204	AGAGAUCU G UAUCAAUA	698	UAUUGAUA UGAUGGCAUGCACUAUGCGCG AGAUCUCU	2120
217	AAUAAAAU G CUAAAUCA	699	UGAUUUAG UGAUGGCAUGCACUAUGCGCG AUUUUAUU	2121
227	UAAAUCAU G AAAAUGUA	700	UACAUUUU UGAUGGCAUGCACUAUGCGCG AUGAUUUA	2122
233	AUGAAAAU G UAGUAAAA	701	UUUUACUA UGAUGGCAUGCACUAUGCGCG AUUUUCAU	2123
294	GGAGUACU G UAGUGGAG	702	CUCCACUA UGAUGGCAUGCACUAUGCGCG AGUACUCC	2124
314	AGCUUUUU G ACAGAAUA	703	UAUUCUGU UGAUGGCAUGCACUAUGCGCG AAAAAGCU	2125
340	AUAGGCAU G CCUGAACC	704	GGUUCAGG UGAUGGCAUGCACUAUGCGCG AUGCCUAU	2126
344	GCAUGCCU G AACCAGAU	705	AUCUGGUU UGAUGGCAUGCACUAUGCGCG AGGCAUGC	2127
353	AACCAGAU G CUCAGAGA	706	UCUCUGAG UGAUGGCAUGCACUAUGCGCG AUCUGGUU	2128
397	GUUUAUCU G CAUGGUAU	707	AUACCAUG UGAUGGCAUGCACUAUGCGCG AGAUAAAC	2129
445	AAUCUUCU G UUGGAUGA	708	UCAUCCAA UGAUGGCAUGCACUAUGCGCG AGAAGAUU	2130
452	UGUUGGAU G AAAGGGAU	709	AUCCCUUU UGAUGGCAUGCACUAUGCGCG AUCCAACA	2131
515	AUAAUCGU G AGCGUUUG	710	CAAACGCU UGAUGGCAUGCACUAUGCGCG ACGAUUAU	2132
523	GAGCGUUU G UUGAACAA	711	UUGUUCAA UGAUGGCAUGCACUAUGCGCG AAACGCUC	2133
526	CGUUUGUU G AACAAGAU	712	AUCUUGUU UGAUGGCAUGCACUAUGCGCG AACAAACG	2134
535	AACAAGAU G UGUGGUAC	713	GUACCACA UGAUGGCAUGCACUAUGCGCG AUCUUGUU	2135
537	CAAGAUGU G UGGUACUU	714	AAGUACCA UGAUGGCAUGCACUAUGCGCG ACAUCUUG	2136
554	UACCAUAU G UUGCUCCA	715	UGGAGCAA UGAUGGCAUGCACUAUGCGCG AUAUGGUA	2137
557	CAUAUGUU G CUCCAGAA	716	UUCUGGAG UGAUGGCAUGCACUAUGCGCG AACAUAUG	2138
571	GAACUUCU G AAGAGAAG	717	CUUCUCUU UGAUGGCAUGCACUAUGCGCG AGAAGUUC	2139
590	AAUUUCAU G CAGAACCA	718	UGGUUCUG UGAUGGCAUGCACUAUGCGCG AUGAAAUU	2140
602	AACCAGUU G AUGUUUGG	719	CCAAACAU UGAUGGCAUGCACUAUGCGCG AACUGGUU	2141
605	CAGUUGAU G UUUGGUCC	720	GGACCAAA UGAUGGCAUGCACUAUGCGCG AUCAACUG	2142
615	UUGGUCCU G UGGAAUAG	721	CUAUUCCA UGAUGGCAUGCACUAUGCGCG AGGACCAA	2143
632	UACUUACU G CAAUGCUC	722	GAGCAUUG UGAUGGCAUGCACUAUGCGCG AGUAAGUA	2144
637	ACUGCAAU G CUCGCUGG	723	CCAGCGAG UGAUGGCAUGCACUAUGCGCG AUUGCAGU	2145
641	CAAUGCUC G CUGGAGAA	724	UUCUCCAG UGAUGGCAUGCACUAUGCGCG GAGCAUUG	2146
652	GGAGAAUU G CCAUGGGA	725	UCCCAUGG UGAUGGCAUGCACUAUGCGCG AAUUCUCC	2147
671	AACCCAGU G ACAGCUGU	726	ACAGCUGU UGAUGGCAUGCACUAUGCGCG ACUGGGUU	2148

678	UGACAGCU G UCAGGAGU	727	ACUCCUGA UGAUGGCAUGCACUAUGCGCG AGCUGUCA	2149
692	AGUAUUCU G ACUGGAAA	728	UUUCCAGU UGAUGGCAUGCACUAUGCGCG AGAAUACU	2150
737	AAAAAAUC G AUUCUGCU	729	AGCAGAAU UGAUGGCAUGCACUAUGCGCG GAUUUUUU	2151
743	UCGAUUCU G CUCCUCUA	730	UAGAGGAG UGAUGGCAUGCACUAUGCGCG AGAAUCGA	2152
757	CUAGCUCU G CUGCAUAA	731	UUAUGCAG UGAUGGCAUGCACUAUGCGCG AGAGCUAG	2153
760	GCUCUGCU G CAUAAAAU	732	AUUUUAUG UGAUGGCAUGCACUAUGCGCG AGCAGAGC	2154
776	UCUUAGUU G AGAAUCCA	733	UGGAUUCU UGAUGGCAUGCACUAUGCGCG AACUAAGA	2155
864	AAGGCCCC G AGUCACUU	734	AAGUGACU UGAUGGCAUGCACUAUGCGCG GGGGCCUU	2156
881	CAGGUGGU G UGUCAGAG	735	CUCUGACA UGAUGGCAUGCACUAUGCGCG ACCACCUG	2157
883	GGUGGUGU G UCAGAGUC	736	GACUCUGA UGAUGGCAUGCACUAUGCGCG ACACCACC	2158
950	UAAACAGU G CUUCUAGU	737	ACUAGAAG UGAUGGCAUGCACUAUGCGCG ACUGUUUA	2159
959	CUUCUAGU G AAGAAAAU	738	AUUUUCUU UGAUGGCAUGCACUAUGCGCG ACUAGAAG	2160
968	AAGAAAAU G UGAAGUAC	739	GUACUUCA UGAUGGCAUGCACUAUGCGCG AUUUUCUU	2161
970	GAAAAUGU G AAGUACUC	740	GAGUACUU UGAUGGCAUGCACUAUGCGCG ACAUUUUC	2162
999	AGAACCCC G CACAGGUC	741	GACCUGUG UGAUGGCAUGCACUAUGCGCG GGGGUUCU	2163
1040	CAUACAUU G AUAAAUUG	742	CAAUUUAU UGAUGGCAUGCACUAUGCGCG AAUGUAUG	2164
1080	GCCCACAU G UCCUGAUC	743	GAUCAGGA UGAUGGCAUGCACUAUGCGCG AUGUGGGC	2165
1085	CAUGUCCU G AUCAUAUG	744	CAUAUGAU UGAUGGCAUGCACUAUGCGCG AGGACAUG	2166
1093	GAUCAUAU G CUUUUGAA	745	UUCAAAAG UGAUGGCAUGCACUAUGCGCG AUAUGAUC	2167
1099	AUGCUUUU G AAUAGUCA	746	UGACUAUU UGAUGGCAUGCACUAUGCGCG AAAAGCAU	2168
1165	AAAAGAAU G ACACGAUU	747	AAUCGUGU UGAUGGCAUGCACUAUGCGCG AUUCUUUU	2169
1170	AAUGACAC G AUUCUUUA	748	UAAAGAAU UGAUGGCAUGCACUAUGCGCG GUGUCAUU	2170
1190	AAUUGGAU G CAGACAAA	749	UUUGUCUG UGAUGGCAUGCACUAUGCGCG AUCCAAUU	2171
1209	UUAUCAAU G CCUGAAAG	750	CUUUCAGG UGAUGGCAUGCACUAUGCGCG AUUGAUAA	2172
1213	CAAUGCCU G AAAGAGAC	751	GUCUCUUU UGAUGGCAUGCACUAUGCGCG AGGCAUUG	2173
1224	AGAGACUU G UGAGAAGU	752	ACUUCUCA UGAUGGCAUGCACUAUGCGCG AAGUCUCU	2174
1226	AGACUUGU G AGAAGUUG	753	CAACUUCU UGAUGGCAUGCACUAUGCGCG ACAAGUCU	2175
1257	GAAAAGUU G UAUGAAUC	754	GAUUCAUA UGAUGGCAUGCACUAUGCGCG AACUUUUC	2176
1261	AGUUGUAU G AAUCAGGU	755	ACCUGAUU UGAUGGCAUGCACUAUGCGCG AUACAACU	2177
1286	CAACAACU G AUAGGAGA	756	UCUCCUAU UGAUGGCAUGCACUAUGCGCG AGUUGUUG	2178
1318	UUCAAAGU G AAUUUGUU	757	AACAAAUU UGAUGGCAUGCACUAUGCGCG ACUUUGAA	2179
1324	GUGAAUUU G UUAGAAAU	758	AUUUCUAA UGAUGGCAUGCACUAUGCGCG AAAUUCAC	2180
1337	AAAUGGAU G AUAAAAUA	759	UAUUUUAU UGAUGGCAUGCACUAUGCGCG AUCCAUUU	2181
1352	UAUUGGUU G ACUUCCGG	760	CCGGAAGU UGAUGGCAUGCACUAUGCGCG AACCAAUA	2182
1373	CUAAGGGU G AUGGAUUG	761	CAAUCCAU UGAUGGCAUGCACUAUGCGCG ACCCUUAG	2183
1402	CACUUCCU G AAGAUUAA	762	UUAAUCUU UGAUGGCAUGCACUAUGCGCG AGGAAGUG	2184
1420	GGGAAGCU G AUUGAUAU	763	AUAUCAAU UGAUGGCAUGCACUAUGCGCG AGCUUCCC	2185
1424	AGCUGAUU G AUAUUGUG	764	CACAAUAU UGAUGGCAUGCACUAUGCGCG AAUCAGCU	2186
1430	UUGAUAUU G UGAGCAGC	765	GCUGCUCA UGAUGGCAUGCACUAUGCGCG AAUAUCAA	2187
1432	GAUAUUGU G AGCAGCCA	766	UGGCUGCU UGAUGGCAUGCACUAUGCGCG ACAAUAUC	2188
1457	GGCUUCCU G CCACAUGA	767	UCAUGUGG UGAUGGCAUGCACUAUGCGCG AGGAAGCC	2189
1464	UGCCACAU G AUCGGACC	768	GGUCCGAU UGAUGGCAUGCACUAUGCGCG AUGUGGCA	2190
1495	AUCCUGGU G AAUAUAGU	769	ACUAUAUU UGAUGGCAUGCACUAUGCGCG ACCAGGAU	2191
1504	AAUAUAGU G CUGCUAUG	770	CAUAGCAG UGAUGGCAUGCACUAUGCGCG ACUAUAUU	2192
1507	AUAGUGCU G CUAUGUUG	771	CAACAUAG UGAUGGCAUGCACUAUGCGCG AGCACUAU	2193
1512	GCUGCUAU G UUGACAUU	772	AAUGUCAA UGAUGGCAUGCACUAUGCGCG AUAGCAGC	2194
1515	GCUAUGUU G ACAUUAUU	773	AAUAAUGU UGAUGGCAUGCACUAUGCGCG AACAUAGC	2195
1545	AUUAUCCU G UCCUGCAA	774	UUGCAGGA UGAUGGCAUGCACUAUGCGCG AGGAUAAU	2196
1550	CCUGUCCU G CAAACUGC	775	GCAGUUUG UGAUGGCAUGCACUAUGCGCG AGGACAGG	2197

1557	UGCAAACU G CAAAUAGU	776	ACUAUUUG UGAUGGCAUGCACUAUGCGCG AGUUUGCA	2198
1573	UAGUUCCU G AAGUGUUC	777	GAACACUU UGAUGGCAUGCACUAUGCGCG AGGAACUA	2199
1578	CCUGAAGU G UUCACUUC	778	GAAGUGAA UGAUGGCAUGCACUAUGCGCG ACUUCAGG	2200
1590	ACUUCCCU G UUUAUCCA	779	UGGAUAAA UGAUGGCAUGCACUAUGCGCG AGGGAAGU	2201
1619	UUUAUUUU G UUUGUUCG	780	CGAACAAA UGAUGGCAUGCACUAUGCGCG AAAAUAAA	2202
1623	UUUUGUUU G UUCGGCAU	781	AUGCCGAA UGAUGGCAUGCACUAUGCGCG AAACAAAA	2203
1656	UCUUAAUU G UAAGCAAA	782	UUUGCUUA UGAUGGCAUGCACUAUGCGCG AAUUAAGA	2204
1681	GAAAGGAU G AAUAGAAU	783	AUUCUAUU UGAUGGCAUGCACUAUGCGCG AUCCUUUC	2205
1696	AUUCAUUU G AUUAUUUC	784	GAAAUAAU UGAUGGCAUGCACUAUGCGCG AAAUGAAU	2206
1710	UUCUUCAU G UGUGUUUA	785	UAAACACA UGAUGGCAUGCACUAUGCGCG AUGAAGAA	2207
1712	CUUCAUGU G UGUUUAGU	786	ACUAAACA UGAUGGCAUGCACUAUGCGCG ACAUGAAG	2208
1714	UCAUGUGU G UUUAGUAU	787	AUACUAAA UGAUGGCAUGCACUAUGCGCG ACACAUGA	2209
1725	UAGUAUCU G AAUUUGAA	788	UUCAAAUU UGAUGGCAUGCACUAUGCGCG AGAUACUA	2210
1731	CUGAAUUU G AAACUCAU	789	AUGAGUUU UGAUGGCAUGCACUAUGCGCG AAAUUCAG	2211
1768	GGGGACAU G AGUUUUCC	790	GGAAAACU UGAUGGCAUGCACUAUGCGCG AUGUCCCC	2212

Input Sequence = AF016582. Cut Site = YG/M or UG/U.
Stem Length = 8. Core Sequence = UGAUG GCAUGCACUAUGC GCG
AF016582 (Homo sapiens checkpoint kinase Chk1 (CHK1) mRNA; 1821 bp)

Table VI: Human Chk1 Zinzyme Ribozyme and Substrate Sequence

Pos	Substrate	Seq ID	Ribozyme	Rz Seq ID
10	GCCGGACA G UCCGCCGA	791	UCGGCGGA GCCGAAAGGCGAGUCAAGGUCU UGUCCGGC	2213
14	GACAGUCC G CCGAGGUG	792	CACCUCGG GCCGAAAGGCGAGUCAAGGUCU GGACUGUC	2214
20	CCGCCGAG G UGCUCGGU	793	ACCGAGCA GCCGAAAGGCGAGUCAAGGUCU CUCGGCGG	2215
22	GCCGAGGU G CUCGGUGG	794	CCACCGAG GCCGAAAGGCGAGUCAAGGUCU ACCUCGGC	2216
27	GGUGCUCG G UGGAGUCA	795	UGACUCCA GCCGAAAGGCGAGUCAAGGUCU CGAGCACC	2217
32	UCGGUGGA G UCAUGGCA	796	UGCCAUGA GCCGAAAGGCGAGUCAAGGUCU UCCACCGA	2218
38	GAGUCAUG G CAGUGCCC	797	GGGCACUG GCCGAAAGGCGAGUCAAGGUCU CAUGACUC	2219
41	UCAUGGCA G UGCCCUUU	798	AAAGGGCA GCCGAAAGGCGAGUCAAGGUCU UGCCAUGA	2220
43	AUGGCAGU G CCCUUUGU	799	ACAAAGGG GCCGAAAGGCGAGUCAAGGUCU ACUGCCAU	2221
50	UGCCCUUU G UGGAAGAC	800	GUCUUCCA GCCGAAAGGCGAGUCAAGGUCU AAAGGGCA	2222
68	GGGACUUG G UGCAAACC	801	GGUUUGCA GCCGAAAGGCGAGUCAAGGUCU CAAGUCCC	2223
70	GACUUGGU G CAAACCCU	802	AGGGUUUG GCCGAAAGGCGAGUCAAGGUCU ACCAAGUC	2224
87	GGGAGAAG G UGCCUAUG	803	CAUAGGCA GCCGAAAGGCGAGUCAAGGUCU CUUCUCCC	2225
89	GAGAAGGU G CCUAUGGA	804	UCCAUAGG GCCGAAAGGCGAGUCAAGGUCU ACCUUCUC	2226
101	AUGGAGAA G UUCAACUU	805	AAGUUGAA GCCGAAAGGCGAGUCAAGGUCU UUCUCCAU	2227
110	UUCAACUU G CUGUGAAU	806	AUUCACAG GCCGAAAGGCGAGUCAAGGUCU AAGUUGAA	2228
113	AACUUGCU G UGAAUAGA	807	UCUAUUCA GCCGAAAGGCGAGUCAAGGUCU AGCAAGUU	2229
122	UGAAUAGA G UAACUGAA	808	UUCAGUUA GCCGAAAGGCGAGUCAAGGUCU UCUAUUCA	2230
134	CUGAAGAA G CAGUCGCA	809	UGCGACUG GCCGAAAGGCGAGUCAAGGUCU UUCUUCAG	2231
137	AAGAAGCA G UCGCAGUG	810	CACUGCGA GCCGAAAGGCGAGUCAAGGUCU UGCUUCUU	2232
140	AAGCAGUC G CAGUGAAG	811	CUUCACUG GCCGAAAGGCGAGUCAAGGUCU GACUGCUU	2233
143	CAGUCGCA G UGAAGAUU	812	AAUCUUCA GCCGAAAGGCGAGUCAAGGUCU UGCGACUG	2234
152	UGAAGAUU G UAGAUAUG	813	CAUAUCUA GCCGAAAGGCGAGUCAAGGUCU AAUCUUCA	2235
163	GAUAUGAA G CGUGCCGU	814	ACGGCACG GCCGAAAGGCGAGUCAAGGUCU UUCAUAUC	2236
165	UAUGAAGC G UGCCGUAG	815	CUACGGCA GCCGAAAGGCGAGUCAAGGUCU GCUUCAUA	2237
167	UGAAGCGU G CCGUAGAC	816	GUCUACGG GCCGAAAGGCGAGUCAAGGUCU ACGCUUCA	2238
170	AGCGUGCC G UAGACUGU	817	ACAGUCUA GCCGAAAGGCGAGUCAAGGUCU GGCACGCU	2239
177	CGUAGACU G UCCAGAAA	818	UUUCUGGA GCCGAAAGGCGAGUCAAGGUCU AGUCUACG	2240
204	AGAGAUCU G UAUCAAUA	819	UAUUGAUA GCCGAAAGGCGAGUCAAGGUCU AGAUCUCU	2241
217	AAUAAAAU G CUAAAUCA	820	UGAUUUAG GCCGAAAGGCGAGUCAAGGUCU AUUUUAUU	2242
233	AUGAAAAU G UAGUAAAA	821	UUUUACUA GCCGAAAGGCGAGUCAAGGUCU AUUUUCAU	2243
236	AAAAUGUA G UAAAAUUC	822	GAAUUUUA GCCGAAAGGCGAGUCAAGGUCU UACAUUUU	2244
249	AUUCUAUG G UCACAGGA	823	UCCUGUGA GCCGAAAGGCGAGUCAAGGUCU CAUAGAAU	2245
264	GAGAGAAG G CAAUAUCC	824	GGAUAUUG GCCGAAAGGCGAGUCAAGGUCU CUUCUCUC	2246
289	UUUCUGGA G UACUGUAG	825	CUACAGUA GCCGAAAGGCGAGUCAAGGUCU UCCAGAAA	2247
294	GGAGUACU G UAGUGGAG	826	CUCCACUA GCCGAAAGGCGAGUCAAGGUCU AGUACUCC	2248
297	GUACUGUA G UGGAGGAG	827	CUCCUCCA GCCGAAAGGCGAGUCAAGGUCU UACAGUAC	2249
307	GGAGGAGA G CUUUUUGA	828	UCAAAAAG GCCGAAAGGCGAGUCAAGGUCU UCUCCUCC	2250
325	AGAAUAGA G CCAGACAU	829	AUGUCUGG GCCGAAAGGCGAGUCAAGGUCU UCUAUUCU	2251
336	AGACAUAG G CAUGCCUG	830	CAGGCAUG GCCGAAAGGCGAGUCAAGGUCU CUAUGUCU	2252
340	AUAGGCAU G CCUGAACC	831	GGUUCAGG GCCGAAAGGCGAGUCAAGGUCU AUGCCUAU	2253
353	AACCAGAU G CUCAGAGA	832	UCUCUGAG GCCGAAAGGCGAGUCAAGGUCU AUCUGGUU	2254
380	AACUCAUG G CAGGGGUG	833	CACCCCUG GCCGAAAGGCGAGUCAAGGUCU CAUGAGUU	2255
386	UGGCAGGG G UGGUUUAU	834	AUAAACCA GCCGAAAGGCGAGUCAAGGUCU CCCUGCCA	2256
389	CAGGGGUG G UUUAUCUG	835	CAGAUAAA GCCGAAAGGCGAGUCAAGGUCU CACCCCUG	2257

397	GUUUAUCU G CAUGGUAU	836	AUACCAUG GCCGAAAGGCGAGUCAAGGUCU AGAUAAAC	2258
402	UCUGCAUG G UAUUGGAA	837	UUCCAAUA GCCGAAAGGCGAGUCAAGGUCU CAUGCAGA	2259
445	AAUCUUCU G UUGGAUGA	838	UCAUCCAA GCCGAAAGGCGAGUCAAGGUCU AGAAGAUU	2260
483	AGACUUUG G CUUGGCAA	839	UUGCCAAG GCCGAAAGGCGAGUCAAGGUCU CAAAGUCU	2261
488	UUGGCUUG G CAACAGUA	840	UACUGUUG GCCGAAAGGCGAGUCAAGGUCU CAAGCCAA	2262
494	UGGCAACA G UAUUUCGG	841	CCGAAAUA GCCGAAAGGCGAGUCAAGGUCU UGUUGCCA	2263
502	GUAUUUCG G UAUAAUAA	842	UUAUUAUA GCCGAAAGGCGAGUCAAGGUCU CGAAAUAC	2264
513	UAAUAAUC G UGAGCGUU	843	AACGCUCA GCCGAAAGGCGAGUCAAGGUCU GAUUAUUA	2265
517	AAUCGUGA G CGUUUGUU	844	AACAAACG GCCGAAAGGCGAGUCAAGGUCU UCACGAUU	2266
519	UCGUGAGC G UUUGUUGA	845	UCAACAAA GCCGAAAGGCGAGUCAAGGUCU GCUCACGA	2267
523	GAGCGUUU G UUGAACAA	846	UUGUUCAA GCCGAAAGGCGAGUCAAGGUCU AAACGCUC	2268
535	AACAAGAU G UGUGGUAC	847	GUACCACA GCCGAAAGGCGAGUCAAGGUCU AUCUUGUU	2269
537	CAAGAUGU G UGGUACUU	848	AAGUACCA GCCGAAAGGCGAGUCAAGGUCU ACAUCUUG	2270
540	GAUGUGUG G UACUUUAC	849	GUAAAGUA GCCGAAAGGCGAGUCAAGGUCU CACACAUC	2271
554	UACCAUAU G UUGCUCCA	850	UGGAGCAA GCCGAAAGGCGAGUCAAGGUCU AUAUGGUA	2272
557	CAUAUGUU G CUCCAGAA	851	UUCUGGAG GCCGAAAGGCGAGUCAAGGUCU AACAUAUG	2273
590	AAUUUCAU G CAGAACCA	852	UGGUUCUG GCCGAAAGGCGAGUCAAGGUCU AUGAAAUU	2274
599	CAGAACCA G UUGAUGUU	853	AACAUCAA GCCGAAAGGCGAGUCAAGGUCU UGGUUCUG	2275
605	CAGUUGAU G UUUGGUCC	854	GGACCAAA GCCGAAAGGCGAGUCAAGGUCU AUCAACUG	2276
610	GAUGUUUG G UCCUGUGG	855	CCACAGGA GCCGAAAGGCGAGUCAAGGUCU CAAACAUC	2277
615	UUGGUCCU G UGGAAUAG	856	CUAUUCCA GCCGAAAGGCGAGUCAAGGUCU AGGACCAA	2278
623	GUGGAAUA G UACUUACU	857	AGUAAGUA GCCGAAAGGCGAGUCAAGGUCU UAUUCCAC	2279
632	UACUUACU G CAAUGCUC	858	GAGCAUUG GCCGAAAGGCGAGUCAAGGUCU AGUAAGUA	2280
637	ACUGCAAU G CUCGCUGG	859	CCAGCGAG GCCGAAAGGCGAGUCAAGGUCU AUUGCAGU	2281
641	CAAUGCUC G CUGGAGAA	860	UUCUCCAG GCCGAAAGGCGAGUCAAGGUCU GAGCAUUG	2282
652	GGAGAAUU G CCAUGGGA	861	UCCCAUGG GCCGAAAGGCGAGUCAAGGUCU AAUUCUCC	2283
669	CCAACCCA G UGACAGCU	862	AGCUGUCA GCCGAAAGGCGAGUCAAGGUCU UGGGUUGG	2284
675	CAGUGACA G CUGUCAGG	863	CCUGACAG GCCGAAAGGCGAGUCAAGGUCU UGUCACUG	2285
678	UGACAGCU G UCAGGAGU	864	ACUCCUGA GCCGAAAGGCGAGUCAAGGUCU AGCUGUCA	2286
685	UGUCAGGA G UAUUCUGA	865	UCAGAAUA GCCGAAAGGCGAGUCAAGGUCU UCCUGACA	2287
743	UCGAUUCU G CUCCUCUA	866	UAGAGGAG GCCGAAAGGCGAGUCAAGGUCU AGAAUCGA	2288
752	CUCCUCUA G CUCUGCUG	867	CAGCAGAG GCCGAAAGGCGAGUCAAGGUCU UAGAGGAG	2289
757	CUAGCUCU G CUGCAUAA	868	UUAUGCAG GCCGAAAGGCGAGUCAAGGUCU AGAGCUAG	2290
760	GCUCUGCU G CAUAAAAU	869	AUUUUAUG GCCGAAAGGCGAGUCAAGGUCU AGCAGAGC	2291
773	AAAUCUUA G UUGAGAAU	870	AUUCUCAA GCCGAAAGGCGAGUCAAGGUCU UAAGAUUU	2292
788	AUCCAUCA G CAAGAAUU	871	AAUUCUUG GCCGAAAGGCGAGUCAAGGUCU UGAUGGAU	2293
826	GAUAGAUG G UACAACAA	872	UUGUUGUA GCCGAAAGGCGAGUCAAGGUCU CAUCUAUC	2294
851	AGAAAGGG G CAAAAAGG	873	CCUUUUUG GCCGAAAGGCGAGUCAAGGUCU CCCUUUCU	2295
859	GCAAAAAG G CCCCGAGU	874	ACUCGGGG GCCGAAAGGCGAGUCAAGGUCU CUUUUUGC	2296
866	GGCCCCGA G UCACUUCA	875	UGAAGUGA GCCGAAAGGCGAGUCAAGGUCU UCGGGGCC	2297
876	CACUUCAG G UGGUGUGU	876	ACACACCA GCCGAAAGGCGAGUCAAGGUCU CUGAAGUG	2298
879	UUCAGGUG G UGUGUCAG	877	CUGACACA GCCGAAAGGCGAGUCAAGGUCU CACCUGAA	2299
881	CAGGUGGU G UGUCAGAG	878	CUCUGACA GCCGAAAGGCGAGUCAAGGUCU ACCACCUG	2300
883	GGUGGUGU G UCAGAGUC	879	GACUCUGA GCCGAAAGGCGAGUCAAGGUCU ACACCACC	2301
889	GUGUCAGA G UCUCCCAG	880	CUGGGAGA GCCGAAAGGCGAGUCAAGGUCU UCUGACAC	2302
897	GUCUCCCA G UGGAUUUU	881	AAAAUCCA GCCGAAAGGCGAGUCAAGGUCU UGGGAGAC	2303
910	UUUUCUAA G CACAUUCA	882	UGAAUGUG GCCGAAAGGCGAGUCAAGGUCU UUAGAAAA	2304
941	UCUCUCCA G UAAACAGU	883	ACUGUUUA GCCGAAAGGCGAGUCAAGGUCU UGGAGAGA	2305
948	AGUAAACA G UGCUUCUA	884	UAGAAGCA GCCGAAAGGCGAGUCAAGGUCU UGUUUACU	2306

950	UAAACAGU G CUUCUAGU	885	ACUAGAAG GCCGAAAGGCGAGUCAAGGUCU ACUGUUUA	2307
957	UGCUUCUA G UGAAGAAA	886	UUUCUUCA GCCGAAAGGCGAGUCAAGGUCU UAGAAGCA	2308
968	AAGAAAAU G UGAAGUAC	887	GUACUUCA GCCGAAAGGCGAGUCAAGGUCU AUUUUCUU	2309
973	AAUGUGAA G UACUCCAG	888	CUGGAGUA GCCGAAAGGCGAGUCAAGGUCU UUCACAUU	2310
981	GUACUCCA G UUCUCAGC	889	GCUGAGAA GCCGAAAGGCGAGUCAAGGUCU UGGAGUAC	2311
988	AGUUCUCA G CCAGAACC	890	GGUUCUGG GCCGAAAGGCGAGUCAAGGUCU UGAGAACU	2312
999	AGAACCCC G CACAGGUC	891	GACCUGUG GCCGAAAGGCGAGUCAAGGUCU GGGGUUCU	2313
1005	CCGCACAG G UCUUUCCU	892	AGGAAAGA GCCGAAAGGCGAGUCAAGGUCU CUGUGCGG	2314
1026	GGAUACCA G CCCCUCAU	893	AUGAGGGG GCCGAAAGGCGAGUCAAGGUCU UGGUAUCC	2315
1049	AUAAAUUG G UACAAGGG	894	CCCUUGUA GCCGAAAGGCGAGUCAAGGUCU CAAUUUAU	2316
1062	AGGGAUCA G CUUUUCCC	895	GGGAAAAG GCCGAAAGGCGAGUCAAGGUCU UGAUCCCU	2317
1072	UUUUCCCA G CCCACAUG	896	CAUGUGGG GCCGAAAGGCGAGUCAAGGUCU UGGGAAAA	2318
1080	GCCCACAU G UCCUGAUC	897	GAUCAGGA GCCGAAAGGCGAGUCAAGGUCU AUGUGGGC	2319
1093	GAUCAUAU G CUUUUGAA	898	UUCAAAAG GCCGAAAGGCGAGUCAAGGUCU AUAUGAUC	2320
1104	UUUGAAUA G UCAGUUAC	899	GUAACUGA GCCGAAAGGCGAGUCAAGGUCU UAUUCAAA	2321
1108	AAUAGUCA G UUACUUGG	900	CCAAGUAA GCCGAAAGGCGAGUCAAGGUCU UGACUAUU	2322
1116	GUUACUUG G CACCCCAG	901	CUGGGGUG GCCGAAAGGCGAGUCAAGGUCU CAAGUAAC	2323
1144	AACCCCUG G CAGCGGUU	902	AACCGCUG GCCGAAAGGCGAGUCAAGGUCU CAGGGGUU	2324
1147	CCCUGGCA G CGGUUGGU	903	ACCAACCG GCCGAAAGGCGAGUCAAGGUCU UGCCAGGG	2325
1150	UGGCAGCG G UUGGUCAA	904	UUGACCAA GCCGAAAGGCGAGUCAAGGUCU CGCUGCCA	2326
1154	AGCGGUUG G UCAAAAGA	905	UCUUUUGA GCCGAAAGGCGAGUCAAGGUCU CAACCGCU	2327
1190	AAUUGGAU G CAGACAAA	906	UUUGUCUG GCCGAAAGGCGAGUCAAGGUCU AUCCAAUU	2328
1209	UUAUCAAU G CCUGAAAG	907	CUUUCAGG GCCGAAAGGCGAGUCAAGGUCU AUUGAUAA	2329
1224	AGAGACUU G UGAGAAGU	908	ACUUCUCA GCCGAAAGGCGAGUCAAGGUCU AAGUCUCU	2330
1231	UGUGAGAA G UUGGGCUA	909	UAGCCCAA GCCGAAAGGCGAGUCAAGGUCU UUCUCACA	2331
1236	GAAGUUGG G CUAUCAAU	910	AUUGAUAG GCCGAAAGGCGAGUCAAGGUCU CCAACUUC	2332
1254	GAAGAAAA G UUGUAUGA	911	UCAUACAA GCCGAAAGGCGAGUCAAGGUCU UUUUCUUC	2333
1257	GAAAAGUU G UAUGAAUC	912	GAUUCAUA GCCGAAAGGCGAGUCAAGGUCU AACUUUUC	2334
1268	UGAAUCAG G UUACUAUA	913	UAUAGUAA GCCGAAAGGCGAGUCAAGGUCU CUGAUUCA	2335
1316	UUUUCAAA G UGAAUUUG	914	CAAAUUCA GCCGAAAGGCGAGUCAAGGUCU UUUGAAAA	2336
1324	GUGAAUUU G UUAGAAAU	915	AUUUCUAA GCCGAAAGGCGAGUCAAGGUCU AAAUUCAC	2337
1349	AAAUAUUG G UUGACUUC	916	GAAGUCAA GCCGAAAGGCGAGUCAAGGUCU CAAUAUUU	2338
1360	GACUUCCG G CUUUCUAA	917	UUAGAAAG GCCGAAAGGCGAGUCAAGGUCU CGGAAGUC	2339
1371	UUCUAAGG G UGAUGGAU	918	AUCCAUCA GCCGAAAGGCGAGUCAAGGUCU CCUUAGAA	2340
1384	GGAUUGGA G UUCAAGAG	919	CUCUUGAA GCCGAAAGGCGAGUCAAGGUCU UCCAAUCC	2341
1417	AAAGGGAA G CUGAUUGA	920	UCAAUCAG GCCGAAAGGCGAGUCAAGGUCU UUCCCUUU	2342
1430	UUGAUAUU G UGAGCAGC	921	GCUGCUCA GCCGAAAGGCGAGUCAAGGUCU AAUAUCAA	2343
1434	UAUUGUGA G CAGCCAGA	922	UCUGGCUG GCCGAAAGGCGAGUCAAGGUCU UCACAAUA	2344
1437	UGUGAGCA G CCAGAAGG	923	CCUUCUGG GCCGAAAGGCGAGUCAAGGUCU UGCUCACA	2345
1445	GCCAGAAG G UUUGGCUU	924	AAGCCAAA GCCGAAAGGCGAGUCAAGGUCU CUUCUGGC	2346
1450	AAGGUUUG G CUUCCUGC	925	GCAGGAAG GCCGAAAGGCGAGUCAAGGUCU CAAACCUU	2347
1457	GGCUUCCU G CCACAUGA	926	UCAUGUGG GCCGAAAGGCGAGUCAAGGUCU AGGAAGCC	2348
1477	GACCAUCG G CUCUGGGG	927	CCCCAGAG GCCGAAAGGCGAGUCAAGGUCU CGAUGGUC	2349
1493	GAAUCCUG G UGAAUAUA	928	UAUAUUCA GCCGAAAGGCGAGUCAAGGUCU CAGGAUUC	2350
1502	UGAAUAUA G UGCUGCUA	929	UAGCAGCA GCCGAAAGGCGAGUCAAGGUCU UAUAUUCA	2351
1504	AAUAUAGU G CUGCUAUG	930	CAUAGCAG GCCGAAAGGCGAGUCAAGGUCU ACUAUAUU	2352
1507	AUAGUGCU G CUAUGUUG	931	CAACAUAG GCCGAAAGGCGAGUCAAGGUCU AGCACUAU	2353
1512	GCUGCUAU G UUGACAUU	932	AAUGUCAA GCCGAAAGGCGAGUCAAGGUCU AUAGCAGC	2354
1545	AUUAUCCU G UCCUGCAA	933	UUGCAGGA GCCGAAAGGCGAGUCAAGGUCU AGGAUAAU	2355

1550	CCUGUCCU G CAAACUGC	934	GCAGUUUG GCCGAAAGGCGAGUCAAGGUCU AGGACAGG	2356
1557	UGCAAACU G CAAAUAGU	935	ACUAUUUG GCCGAAAGGCGAGUCAAGGUCU AGUUUGCA	2357
1564	UGCAAAUA G UAGUUCCU	936	AGGAACUA GCCGAAAGGCGAGUCAAGGUCU UAUUUGCA	2358
1567	AAAUAGUA G UUCCUGAA	937	UUCAGGAA GCCGAAAGGCGAGUCAAGGUCU UACUAUUU	2359
1576	UUCCUGAA G UGUUCACU	938	AGUGAACA GCCGAAAGGCGAGUCAAGGUCU UUCAGGAA	2360
1578	CCUGAAGU G UUCACUUC	939	GAAGUGAA GCCGAAAGGCGAGUCAAGGUCU ACUUCAGG	2361
1590	ACUUCCCU G UUUAUCCA	940	UGGAUAAA GCCGAAAGGCGAGUCAAGGUCU AGGGAAGU	2362
1619	UUUAUUUU G UUUGUUCG	941	CGAACAAA GCCGAAAGGCGAGUCAAGGUCU AAAAUAAA	2363
1623	UUUUGUUU G UUCGGCAU	942	AUGCCGAA GCCGAAAGGCGAGUCAAGGUCU AAACAAAA	2364
1628	UUUGUUCG G CAUACAAA	943	UUUGUAUG GCCGAAAGGCGAGUCAAGGUCU CGAACAAA	2365
1656	UCUUAAUU G UAAGCAAA	944	UUUGCUUA GCCGAAAGGCGAGUCAAGGUCU AAUUAAGA	2366
1660	AAUUGUAA G CAAAACUU	945	AAGUUUUG GCCGAAAGGCGAGUCAAGGUCU UUACAAUU	2367
1710	UUCUUCAU G UGUGUUUA	946	UAAACACA GCCGAAAGGCGAGUCAAGGUCU AUGAAGAA	2368
1712	CUUCAUGU G UGUUUAGU	947	ACUAAACA GCCGAAAGGCGAGUCAAGGUCU ACAUGAAG	2369
1714	UCAUGUGU G UUUAGUAU	948	AUACUAAA GCCGAAAGGCGAGUCAAGGUCU ACACAUGA	2370
1719	UGUGUUUA G UAUCUGAA	949	UUCAGAUA GCCGAAAGGCGAGUCAAGGUCU UAAACACA	2371
1743	CUCAUCUG G UGGAAACC	950	GGUUUCCA GCCGAAAGGCGAGUCAAGGUCU CAGAUGAG	2372
1754	GAAACCAA G UUUCAGGG	951	CCCUGAAA GCCGAAAGGCGAGUCAAGGUCU UUGGUUUC	2373
1770	GGACAUGA G UUUUCCAG	952	CUGGAAAA GCCGAAAGGCGAGUCAAGGUCU UCAUGUCC	2374
1778	GUUUUCCA G CUUUUAUA	953	UAUAAAAG GCCGAAAGGCGAGUCAAGGUCU UGGAAAAC	2375
1792	AUACACAC G UAUCUCAU	954	AUGAGAUA GCCGAAAGGCGAGUCAAGGUCU GUGUGUAU	2376

Input Sequence = AF016582. Cut Site = G/Y

Stem Length = 8 . Core Sequence = GCcgaaagGCGaGuCaaGGuCu

AF016582 (Homo sapiens checkpoint kinase Chk1 (CHK1) mRNA; 1821 bp)

Table VII: Human Chk1 DNAzyme and Substrate Sequence

Pos	Substrate	Seq ID	DNAzyme	Seq ID
10	GCCGGACA G UCCGCCGA	791	TCGGCGGA GGCTAGCTACAACGA TGTCCGGC	2377
14	GACAGUCC G CCGAGGUG	792	CACCTCGG GGCTAGCTACAACGA GGACTGTC	2378
20	CCGCCGAG G UGCUCGGU	793	ACCGAGCA GGCTAGCTACAACGA CTCGGCGG	2379
22	GCCGAGGU G CUCGGUGG	794	CCACCGAG GGCTAGCTACAACGA ACCTCGGC	2380
27	GGUGCUCG G UGGAGUCA	795	TGACTCCA GGCTAGCTACAACGA CGAGCACC	2381
32	UCGGUGGA G UCAUGGCA	796	TGCCATGA GGCTAGCTACAACGA TCCACCGA	2382
38	GAGUCAUG G CAGUGCCC	797	GGGCACTG GGCTAGCTACAACGA CATGACTC	2383
41	UCAUGGCA G UGCCCUUU	798	AAAGGGCA GGCTAGCTACAACGA TGCCATGA	2384
43	AUGGCAGU G CCCUUUGU	799	ACAAAGGG GGCTAGCTACAACGA ACTGCCAT	2385
50	UGCCCUUU G UGGAAGAC	800	GTCTTCCA GGCTAGCTACAACGA AAAGGGCA	2386
68	GGGACUUG G UGCAAACC	801	GGTTTGCA GGCTAGCTACAACGA CAAGTCCC	2387
70	GACUUGGU G CAAACCCU	802	AGGGTTTG GGCTAGCTACAACGA ACCAAGTC	2388
87	GGGAGAAG G UGCCUAUG	803	CATAGGCA GGCTAGCTACAACGA CTTCTCCC	2389
89	GAGAAGGU G CCUAUGGA	804	TCCATAGG GGCTAGCTACAACGA ACCTTCTC	2390
101	AUGGAGAA G UUCAACUU	805	AAGTTGAA GGCTAGCTACAACGA TTCTCCAT	2391
110	UUCAACUU G CUGUGAAU	806	ATTCACAG GGCTAGCTACAACGA AAGTTGAA	2392
113	AACUUGCU G UGAAUAGA	807	TCTATTCA GGCTAGCTACAACGA AGCAAGTT	2393
122	UGAAUAGA G UAACUGAA	808	TTCAGTTA GGCTAGCTACAACGA TCTATTCA	2394
134	CUGAAGAA G CAGUCGCA	809	TGCGACTG GGCTAGCTACAACGA TTCTTCAG	2395
137	AAGAAGCA G UCGCAGUG	810	CACTGCGA GGCTAGCTACAACGA TGCTTCTT	2396
140	AAGCAGUC G CAGUGAAG	811	CTTCACTG GGCTAGCTACAACGA GACTGCTT	2397
143	CAGUCGCA G UGAAGAUU	812	AATCTTCA GGCTAGCTACAACGA TGCGACTG	2398
152	UGAAGAUU G UAGAUAUG	813	CATATCTA GGCTAGCTACAACGA AATCTTCA	2399
163	GAUAUGAA G CGUGCCGU	814	ACGGCACG GGCTAGCTACAACGA TTCATATC	2400
165	UAUGAAGC G UGCCGUAG	815	CTACGGCA GGCTAGCTACAACGA GCTTCATA	2401
167	UGAAGCGU G CCGUAGAC	816	GTCTACGG GGCTAGCTACAACGA ACGCTTCA	2402
170	AGCGUGCC G UAGACUGU	817	ACAGTCTA GGCTAGCTACAACGA GGCACGCT	2403
177	CGUAGACU G UCCAGAAA	818	TTTCTGGA GGCTAGCTACAACGA AGTCTACG	2404
204	AGAGAUCU G UAUCAAUA	819	TATTGATA GGCTAGCTACAACGA AGATCTCT	2405
217	AAUAAAAU G CUAAAUCA	820	TGATTTAG GGCTAGCTACAACGA ATTTTATT	2406
233	AUGAAAAU G UAGUAAAA	821	TTTTACTA GGCTAGCTACAACGA ATTTTCAT	2407
236	AAAAUGUA G UAAAAUUC	822	GAATTTTA GGCTAGCTACAACGA TACATTTT	2408
249	AUUCUAUG G UCACAGGA	823	TCCTGTGA GGCTAGCTACAACGA CATAGAAT	2409
264	GAGAGAAG G CAAUAUCC	824	GGATATTG GGCTAGCTACAACGA CTTCTCTC	2410
289	UUUCUGGA G UACUGUAG	825	CTACAGTA GGCTAGCTACAACGA TCCAGAAA	2411
294	GGAGUACU G UAGUGGAG	826	CTCCACTA GGCTAGCTACAACGA AGTACTCC	2412
297	GUACUGUA G UGGAGGAG	827	CTCCTCCA GGCTAGCTACAACGA TACAGTAC	2413
307	GGAGGAGA G CUUUUUGA	828	TCAAAAAG GGCTAGCTACAACGA TCTCCTCC	2414
325	AGAAUAGA G CCAGACAU	829	ATGTCTGG GGCTAGCTACAACGA TCTATTCT	2415
336	AGACAUAG G CAUGCCUG	830	CAGGCATG GGCTAGCTACAACGA CTATGTCT	2416
340	AUAGGCAU G CCUGAACC	831	GGTTCAGG GGCTAGCTACAACGA ATGCCTAT	2417
353	AACCAGAU G CUCAGAGA	832	TCTCTGAG GGCTAGCTACAACGA ATCTGGTT	2418
380	AACUCAUG G CAGGGGUG	833	CACCCCTG GGCTAGCTACAACGA CATGAGTT	2419
386	UGGCAGGG G UGGUUUAU	834	ATAAACCA GGCTAGCTACAACGA CCCTGCCA	2420
389	CAGGGGUG G UUUAUCUG	835	CAGATAAA GGCTAGCTACAACGA CACCCCTG	2421
397	GUUUAUCU G CAUGGUAU	836	ATACCATG GGCTAGCTACAACGA AGATAAAC	2422

402	UCUGCAUG G UAUUGGAA	837	TTCCAATA GGCTAGCTACAACGA CATGCAGA	2423
445	AAUCUUCU G UUGGAUGA	838	TCATCCAA GGCTAGCTACAACGA AGAAGATT	2424
483	AGACUUUG G CUUGGCAA	839	TTGCCAAG GGCTAGCTACAACGA CAAAGTCT	2425
488	UUGGCUUG G CAACAGUA	840	TACTGTTG GGCTAGCTACAACGA CAAGCCAA	2426
494	UGGCAACA G UAUUUCGG	841	CCGAAATA GGCTAGCTACAACGA TGTTGCCA	2427
502	GUAUUUCG G UAUAAUAA	842	TTATTATA GGCTAGCTACAACGA CGAAATAC	2428
513	UAAUAAUC G UGAGCGUU	843	AACGCTCA GGCTAGCTACAACGA GATTATTA	2429
517	AAUCGUGA G CGUUUGUU	844	AACAAACG GGCTAGCTACAACGA TCACGATT	2430
519	UCGUGAGC G UUUGUUGA	845	TCAACAAA GGCTAGCTACAACGA GCTCACGA	2431
523	GAGCGUUU G UUGAACAA	846	TTGTTCAA GGCTAGCTACAACGA AAACGCTC	2432
535	AACAAGAU G UGUGGUAC	847	GTACCACA GGCTAGCTACAACGA ATCTTGTT	2433
537	CAAGAUGU G UGGUACUU	848	AAGTACCA GGCTAGCTACAACGA ACATCTTG	2434
540	GAUGUGUG G UACUUUAC	849	GTAAAGTA GGCTAGCTACAACGA CACACATC	2435
554	UACCAUAU G UUGCUCCA	850	TGGAGCAA GGCTAGCTACAACGA ATATGGTA	2436
557	CAUAUGUU G CUCCAGAA	851	TTCTGGAG GGCTAGCTACAACGA AACATATG	2437
590	AAUUUCAU G CAGAACCA	852	TGGTTCTG GGCTAGCTACAACGA ATGAAATT	2438
599	CAGAACCA G UUGAUGUU	853	AACATCAA GGCTAGCTACAACGA TGGTTCTG	2439
605	CAGUUGAU G UUUGGUCC	854	GGACCAAA GGCTAGCTACAACGA ATCAACTG	2440
610	GAUGUUUG G UCCUGUGG	855	CCACAGGA GGCTAGCTACAACGA CAAACATC	2441
615	UUGGUCCU G UGGAAUAG	856	CTATTCCA GGCTAGCTACAACGA AGGACCAA	2442
623	GUGGAAUA G UACUUACU	857	AGTAAGTA GGCTAGCTACAACGA TATTCCAC	2443
632	UACUUACU G CAAUGCUC	858	GAGCATTG GGCTAGCTACAACGA AGTAAGTA	2444
637	ACUGCAAU G CUCGCUGG	859	CCAGCGAG GGCTAGCTACAACGA ATTGCAGT	2445
641	CAAUGCUC G CUGGAGAA	860	TTCTCCAG GGCTAGCTACAACGA GAGCATTG	2446
652	GGAGAAUU G CCAUGGGA	861	TCCCATGG GGCTAGCTACAACGA AATTCTCC	2447
669	CCAACCCA G UGACAGCU	862	AGCTGTCA GGCTAGCTACAACGA TGGGTTGG	2448
675	CAGUGACA G CUGUCAGG	863	CCTGACAG GGCTAGCTACAACGA TGTCACTG	2449
678	UGACAGCU G UCAGGAGU	864	ACTCCTGA GGCTAGCTACAACGA AGCTGTCA	2450
685	UGUCAGGA G UAUUCUGA	865	TCAGAATA GGCTAGCTACAACGA TCCTGACA	2451
743	UCGAUUCU G CUCCUCUA	866	TAGAGGAG GGCTAGCTACAACGA AGAATCGA	2452
752	CUCCUCUA G CUCUGCUG	867	CAGCAGAG GGCTAGCTACAACGA TAGAGGAG	2453
757	CUAGCUCU G CUGCAUAA	868	TTATGCAG GGCTAGCTACAACGA AGAGCTAG	2454
760	GCUCUGCU G CAUAAAAU	869	ATTTTATG GGCTAGCTACAACGA AGCAGAGC	2455
773	AAAUCUUA G UUGAGAAU	870	ATTCTCAA GGCTAGCTACAACGA TAAGATTT	2456
788	AUCCAUCA G CAAGAAUU	871	AATTCTTG GGCTAGCTACAACGA TGATGGAT	2457
826	GAUAGAUG G UACAACAA	872	TTGTTGTA GGCTAGCTACAACGA CATCTATC	2458
851	AGAAAGGG G CAAAAAGG	873	CCTTTTTG GGCTAGCTACAACGA CCCTTTCT	2459
859	GCAAAAAG G CCCCGAGU	874	ACTCGGGG GGCTAGCTACAACGA CTTTTTGC	2460
866	GGCCCCGA G UCACUUCA	875	TGAAGTGA GGCTAGCTACAACGA TCGGGGCC	2461
876	CACUUCAG G UGGUGUGU	876	ACACACCA GGCTAGCTACAACGA CTGAAGTG	2462
879	UUCAGGUG G UGUGUCAG	877	CTGACACA GGCTAGCTACAACGA CACCTGAA	2463
881	CAGGUGGU G UGUCAGAG	878	CTCTGACA GGCTAGCTACAACGA ACCACCTG	2464
883	GGUGGUGU G UCAGAGUC	879	GACTCTGA GGCTAGCTACAACGA ACACCACC	2465
889	GUGUCAGA G UCUCCCAG	880	CTGGGAGA GGCTAGCTACAACGA TCTGACAC	2466
897	GUCUCCCA G UGGAUUUU	881	AAAATCCA GGCTAGCTACAACGA TGGGAGAC	2467
910	UUUUCUAA G CACAUUCA	882	TGAATGTG GGCTAGCTACAACGA TTAGAAAA	2468
941	UCUCUCCA G UAAACAGU	883	ACTGTTTA GGCTAGCTACAACGA TGGAGAGA	2469
948	AGUAAACA G UGCUUCUA	884	TAGAAGCA GGCTAGCTACAACGA TGTTTACT	2470
950	UAAACAGU G CUUCUAGU	885	ACTAGAAG GGCTAGCTACAACGA ACTGTTTA	2471

957	UGCUUCUA G UGAAGAAA	886	TTTCTTCA GGCTAGCTACAACGA TAGAAGCA	2472
968	AAGAAAAU G UGAAGUAC	887	GTACTTCA GGCTAGCTACAACGA ATTTTCTT	2473
973	AAUGUGAA G UACUCCAG	888	CTGGAGTA GGCTAGCTACAACGA TTCACATT	2474
981	GUACUCCA G UUCUCAGC	889	GCTGAGAA GGCTAGCTACAACGA TGGAGTAC	2475
988	AGUUCUCA G CCAGAACC	890	GGTTCTGG GGCTAGCTACAACGA TGAGAACT	2476
999	AGAACCCC G CACAGGUC	891	GACCTGTG GGCTAGCTACAACGA GGGGTTCT	2477
1005	CCGCACAG G UCUUUCCU	892	AGGAAAGA GGCTAGCTACAACGA CTGTGCGG	2478
1026	GGAUACCA G CCCCUCAU	893	ATGAGGGG GGCTAGCTACAACGA TGGTATCC	2479
1049	AUAAAUUG G UACAAGGG	894	CCCTTGTA GGCTAGCTACAACGA CAATTTAT	2480
1062	AGGGAUCA G CUUUUCCC	895	GGGAAAAG GGCTAGCTACAACGA TGATCCCT	2481
1072	UUUUCCCA G CCCACAUG	896	CATGTGGG GGCTAGCTACAACGA TGGGAAAA	2482
1080	GCCCACAU G UCCUGAUC	897	GATCAGGA GGCTAGCTACAACGA ATGTGGGC	2483
1093	GAUCAUAU G CUUUUGAA	898	TTCAAAAG GGCTAGCTACAACGA ATATGATC	2484
1104	UUUGAAUA G UCAGUUAC	899	GTAACTGA GGCTAGCTACAACGA TATTCAAA	2485
1108	AAUAGUCA G UUACUUGG	900	CCAAGTAA GGCTAGCTACAACGA TGACTATT	2486
1116	GUUACUUG G CACCCCAG	901	CTGGGGTG GGCTAGCTACAACGA CAAGTAAC	2487
1144	AACCCCUG G CAGCGGUU	902	AACCGCTG GGCTAGCTACAACGA CAGGGGTT	2488
1147	CCCUGGCA G CGGUUGGU	903	ACCAACCG GGCTAGCTACAACGA TGCCAGGG	2489
1150	UGGCAGCG G UUGGUCAA	904	TTGACCAA GGCTAGCTACAACGA CGCTGCCA	2490
1154	AGCGGUUG G UCAAAAGA	905	TCTTTTGA GGCTAGCTACAACGA CAACCGCT	2491
1190	AAUUGGAU G CAGACAAA	906	TTTGTCTG GGCTAGCTACAACGA ATCCAATT	2492
1209	UUAUCAAU G CCUGAAAG	907	CTTTCAGG GGCTAGCTACAACGA ATTGATAA	2493
1224	AGAGACUU G UGAGAAGU	908	ACTTCTCA GGCTAGCTACAACGA AAGTCTCT	2494
1231	UGUGAGAA G UUGGGCUA	909	TAGCCCAA GGCTAGCTACAACGA TTCTCACA	2495
1236	GAAGUUGG G CUAUCAAU	910	ATTGATAG GGCTAGCTACAACGA CCAACTTC	2496
1254	GAAGAAAA G UUGUAUGA	911	TCATACAA GGCTAGCTACAACGA TTTTCTTC	2497
1257	GAAAAGUU G UAUGAAUC	912	GATTCATA GGCTAGCTACAACGA AACTTTTC	2498
1268	UGAAUCAG G UUACUAUA	913	TATAGTAA GGCTAGCTACAACGA CTGATTCA	2499
1316	UUUUCAAA G UGAAUUUG	914	CAAATTCA GGCTAGCTACAACGA TTTGAAAA	2500
1324	GUGAAUUU G UUAGAAAU	915	ATTTCTAA GGCTAGCTACAACGA AAATTCAC	2501
1349	AAAUAUUG G UUGACUUC	916	GAAGTCAA GGCTAGCTACAACGA CAATATTT	2502
1360	GACUUCCG G CUUUCUAA	917	TTAGAAAG GGCTAGCTACAACGA CGGAAGTC	2503
1371	UUCUAAGG G UGAUGGAU	918	ATCCATCA GGCTAGCTACAACGA CCTTAGAA	2504
1384	GGAUUGGA G UUCAAGAG	919	CTCTTGAA GGCTAGCTACAACGA TCCAATCC	2505
1417	AAAGGGAA G CUGAUUGA	920	TCAATCAG GGCTAGCTACAACGA TTCCCTTT	2506
1430	UUGAUAUU G UGAGCAGC	921	GCTGCTCA GGCTAGCTACAACGA AATATCAA	2507
1434	UAUUGUGA G CAGCCAGA	922	TCTGGCTG GGCTAGCTACAACGA TCACAATA	2508
1437	UGUGAGCA G CCAGAAGG	923	CCTTCTGG GGCTAGCTACAACGA TGCTCACA	2509
1445	GCCAGAAG G UUUGGCUU	924	AAGCCAAA GGCTAGCTACAACGA CTTCTGGC	2510
1450	AAGGUUUG G CUUCCUGC	925	GCAGGAAG GGCTAGCTACAACGA CAAACCTT	2511
1457	GGCUUCCU G CCACAUGA	926	TCATGTGG GGCTAGCTACAACGA AGGAAGCC	2512
1477	GACCAUCG G CUCUGGGG	927	CCCCAGAG GGCTAGCTACAACGA CGATGGTC	2513
1493	GAAUCCUG G UGAAUAUA	928	TATATTCA GGCTAGCTACAACGA CAGGATTC	2514
1502	UGAAUAUA G UGCUGCUA	929	TAGCAGCA GGCTAGCTACAACGA TATATTCA	2515
1504	AAUAUAGU G CUGCUAUG	930	CATAGCAG GGCTAGCTACAACGA ACTATATT	2516
1507	AUAGUGCU G CUAUGUUG	931	CAACATAG GGCTAGCTACAACGA AGCACTAT	2517
1512	GCUGCUAU G UUGACAUU	932	AATGTCAA GGCTAGCTACAACGA ATAGCAGC	2518
1545	AUUAUCCU G UCCUGCAA	933	TTGCAGGA GGCTAGCTACAACGA AGGATAAT	2519
1550	CCUGUCCU G CAAACUGC	934	GCAGTTTG GGCTAGCTACAACGA AGGACAGG	2520

1557	UGCAAACU G CAAAUAGU	935	ACTATTTG GGCTAGCTACAACGA AGTTTGCA	2521
1564	UGCAAAUA G UAGUUCCU	936	AGGAACTA GGCTAGCTACAACGA TATTTGCA	2522
1567	AAAUAGUA G UUCCUGAA	937	TTCAGGAA GGCTAGCTACAACGA TACTATTT	2523
1576	UUCCUGAA G UGUUCACU	938	AGTGAACA GGCTAGCTACAACGA TTCAGGAA	2524
1578	CCUGAAGU G UUCACUUC	939	GAAGTGAA GGCTAGCTACAACGA ACTTCAGG	2525
1590	ACUUCCCU G UUUAUCCA	940	TGGATAAA GGCTAGCTACAACGA AGGGAAGT	2526
1619	UUUAUUUU G UUUGUUCG	941	CGAACAAA GGCTAGCTACAACGA AAAATAAA	2527
1623	UUUUGUUU G UUCGGCAU	942	ATGCCGAA GGCTAGCTACAACGA AAACAAAA	2528
1628	UUUGUUCG G CAUACAAA	943	TTTGTATG GGCTAGCTACAACGA CGAACAAA	2529
1656	UCUUAAUU G UAAGCAAA	944	TTTGCTTA GGCTAGCTACAACGA AATTAAGA	2530
1660	AAUUGUAA G CAAAACUU	945	AAGTTTTG GGCTAGCTACAACGA TTACAATT	2531
1710	UUCUUCAU G UGUGUUUA	946	TAAACACA GGCTAGCTACAACGA ATGAAGAA	2532
1712	CUUCAUGU G UGUUUAGU	947	ACTAAACA GGCTAGCTACAACGA ACATGAAG	2533
1714	UCAUGUGU G UUUAGUAU	948	ATACTAAA GGCTAGCTACAACGA ACACATGA	2534
1719	UGUGUUUA G UAUCUGAA	949	TTCAGATA GGCTAGCTACAACGA TAAACACA	2535
1743	CUCAUCUG G UGGAAACC	950	GGTTTCCA GGCTAGCTACAACGA CAGATGAG	2536
1754	GAAACCAA G UUUCAGGG	951	CCCTGAAA GGCTAGCTACAACGA TTGGTTTC	2537
1770	GGACAUGA G UUUUCCAG	952	CTGGAAAA GGCTAGCTACAACGA TCATGTCC	2538
1778	GUUUUCCA G CUUUUAUA	953	TATAAAAG GGCTAGCTACAACGA TGGAAAAC	2539
1792	AUACACAC G UAUCUCAU	954	ATGAGATA GGCTAGCTACAACGA GTGTGTAT	2540
35	GUGGAGUC A UGGCAGUG	955	CACTGCCA GGCTAGCTACAACGA GACTCCAC	2541
57	UGUGGAAG A CUGGGACU	956	AGTCCCAG GGCTAGCTACAACGA CTTCCACA	2542
63	AGACUGGG A CUUGGUGC	957	GCACCAAG GGCTAGCTACAACGA CCCAGTCT	2543
74	UGGUGCAA A CCCUGGGA	958	TCCCAGGG GGCTAGCTACAACGA TTGCACCA	2544
93	AGGUGCCU A UGGAGAAG	959	CTTCTCCA GGCTAGCTACAACGA AGGCACCT	2545
106	GAAGUUCA A CUUGCUGU	960	ACAGCAAG GGCTAGCTACAACGA TGAACTTC	2546
117	UGCUGUGA A UAGAGUAA	961	TTACTCTA GGCTAGCTACAACGA TCACAGCA	2547
125	AUAGAGUA A CUGAAGAA	962	TTCTTCAG GGCTAGCTACAACGA TACTCTAT	2548
149	CAGUGAAG A UUGUAGAU	963	ATCTACAA GGCTAGCTACAACGA CTTCACTG	2549
156	GAUUGUAG A UAUGAAGC	964	GCTTCATA GGCTAGCTACAACGA CTACAATC	2550
158	UUGUAGAU A UGAAGCGU	965	ACGCTTCA GGCTAGCTACAACGA ATCTACAA	2551
174	UGCCGUAG A CUGUCCAG	966	CTGGACAG GGCTAGCTACAACGA CTACGGCA	2552
186	UCCAGAAA A UAUUAAGA	967	TCTTAATA GGCTAGCTACAACGA TTTCTGGA	2553
188	CAGAAAAU A UUAAGAAA	968	TTTCTTAA GGCTAGCTACAACGA ATTTTCTG	2554
200	AGAAAGAG A UCUGUAUC	969	GATACAGA GGCTAGCTACAACGA CTCTTTCT	2555
206	AGAUCUGU A UCAAUAAA	970	TTTATTGA GGCTAGCTACAACGA ACAGATCT	2556
210	CUGUAUCA A UAAAAUGC	971	GCATTTTA GGCTAGCTACAACGA TGATACAG	2557
215	UCAAUAAA A UGCUAAAU	972	ATTTAGCA GGCTAGCTACAACGA TTTATTGA	2558
222	AAUGCUAA A UCAUGAAA	973	TTTCATGA GGCTAGCTACAACGA TTAGCATT	2559
225	GCUAAAUC A UGAAAAUG	974	CATTTTCA GGCTAGCTACAACGA GATTTAGC	2560
231	UCAUGAAA A UGUAGUAA	975	TTACTACA GGCTAGCTACAACGA TTTCATGA	2561
241	GUAGUAAA A UUCUAUGG	976	CCATAGAA GGCTAGCTACAACGA TTTACTAC	2562
246	AAAAUUCU A UGGUCACA	977	TGTGACCA GGCTAGCTACAACGA AGAATTTT	2563
252	CUAUGGUC A CAGGAGAG	978	CTCTCCTG GGCTAGCTACAACGA GACCATAG	2564
267	AGAAGGCA A UAUCCAAU	979	ATTGGATA GGCTAGCTACAACGA TGCCTTCT	2565
269	AAGGCAAU A UCCAAUAU	980	ATATTGGA GGCTAGCTACAACGA ATTGCCTT	2566
274	AAUAUCCA A UAUUUAUU	981	AATAAATA GGCTAGCTACAACGA TGGATATT	2567
276	UAUCCAAU A UUUAUUUC	982	GAAATAAA GGCTAGCTACAACGA ATTGGATA	2568
280	CAAUAUUU A UUUCUGGA	983	TCCAGAAA GGCTAGCTACAACGA AAATATTG	2569

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315	GCUUUUUG A CAGAAUAG	985	CTATTCTG GGCTAGCTACAACGA CAAAAAGC	2571
320	UUGACAGA A UAGAGCCA	986	TGGCTCTA GGCTAGCTACAACGA TCTGTCAA	2572
330	AGAGCCAG A CAUAGGCA	987	TGCCTATG GGCTAGCTACAACGA CTGGCTCT	2573
332	AGCCAGAC A UAGGCAUG	988	CATGCCTA GGCTAGCTACAACGA GTCTGGCT	2574
338	ACAUAGGC A UGCCUGAA	989	TTCAGGCA GGCTAGCTACAACGA GCCTATGT	2575
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351	UGAACCAG A UGCUCAGA	991	TCTGAGCA GGCTAGCTACAACGA CTGGTTCA	2577
361	GCUCAGAG A UUCUUCCA	992	TGGAAGAA GGCTAGCTACAACGA CTCTGAGC	2578
369	AUUCUUCC A UCAACUCA	993	TGAGTTGA GGCTAGCTACAACGA GGAAGAAT	2579
373	UUCCAUCA A CUCAUGGC	994	GCCATGAG GGCTAGCTACAACGA TGATGGAA	2580
377	AUCAACUC A UGGCAGGG	995	CCCTGCCA GGCTAGCTACAACGA GAGTTGAT	2581
393	GGUGGUUU A UCUGCAUG	996	CATGCAGA GGCTAGCTACAACGA AAACCACC	2582
399	UUAUCUGC A UGGUAUUG	997	CAATACCA GGCTAGCTACAACGA GCAGATAA	2583
404	UGCAUGGU A UUGGAAUA	998	TATTCCAA GGCTAGCTACAACGA ACCATGCA	2584
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417	AAUAACUC A CAGGGAUA	1001	TATCCCTG GGCTAGCTACAACGA GAGTTATT	2587
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425	ACAGGGAU A UUAAACCA	1003	TGGTTTAA GGCTAGCTACAACGA ATCCCTGT	2589
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552	UUUACCAU A UGUUGCUC	1021	GAGCAACA GGCTAGCTACAACGA ATGGTAAA	2607
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625	GGAAUAGU A CUUACUGC	1028	GCAGTAAG GGCTAGCTACAACGA ACTATTCC	2614
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655	GAAUUGCC A UGGGACCA	1032	TGGTCCCA GGCTAGCTACAACGA GGCAATTC	2618

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693	GUAUUCUG A CUGGAAAG	1037	CTTTCCAG GGCTAGCTACAACGA CAGAATAC	2623
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738	AAAAAUCG A UUCUGCUC	1043	GAGCAGAA GGCTAGCTACAACGA CGATTTTT	2629
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1734	AAUUUGAA A CUCAUCUG	1172	CAGATGAG GGCTAGCTACAACGA TTCAAATT	2758
1738	UGAAACUC A UCUGGUGG	1173	CCACCAGA GGCTAGCTACAACGA GAGTTTCA	2759
1749	UGGUGGAA A CCAAGUUU	1174	AAACTTGG GGCTAGCTACAACGA TTCCACCA	2760
1764	UUCAGGGG A CAUGAGUU	1175	AACTCATG GGCTAGCTACAACGA CCCCTGAA	2761
1766	CAGGGGAC A UGAGUUUU	1176	AAAACTCA GGCTAGCTACAACGA GTCCCCTG	2762
1784	CAGCUUUU A UACACACG	1177	CGTGTGTA GGCTAGCTACAACGA AAAAGCTG	2763
1786	GCUUUUAU A CACACGUA	1178	TACGTGTG GGCTAGCTACAACGA ATAAAAGC	2764
1788	UUUUAUAC A CACGUAUC	1179	GATACGTG GGCTAGCTACAACGA GTATAAAA	2765

1790	UUAUACAC A CGUAUCUC	1180	GAGATACG GGCTAGCTACAACGA GTGTATAA	2766
1794	ACACACGU A UCUCAUUU	1181	AAATGAGA GGCTAGCTACAACGA ACGTGTGT	2767
1799	CGUAUCUC A UUUUUAUC	1182	GATAAAAA GGCTAGCTACAACGA GAGATACG	2768
1805	UCAUUUUU A UCAAAACA	1183	TGTTTTGA GGCTAGCTACAACGA AAAAATGA	2769
1811	UUAUCAAA A CAUUUUGU	1184	ACAAAATG GGCTAGCTACAACGA TTTGATAA	2770
1813	AUCAAAAC A UUUUGUUU	1185	AAACAAAA GGCTAGCTACAACGA GTTTTGAT	2771

Input Sequence = AF016582 Cut Site = R/Y

Stem Length = 8 . Core Sequence = GGCTAGCTACAACGA

AF016582 (Homo sapiens checkpoint kinase Chk1 (CHK1) mRNA; 1821 bp)

Table VIII: Human Chk1 Amberzyme Ribozyme and Substrate Sequence

Pos	Substrate	Seq ID	Ribozyme	Rz Seq ID
10	GCCGGACA G UCCGCCGA	791	UCGGCGGA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UGUCCGGC	2772
14	GACAGUCC G CCGAGGUG	792	CACCUCGG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG GGACUGUC	2773
20	CCGCCGAG G UGCUCGGU	793	ACCGAGCA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CUCGGCGG	2774
22	GCCGAGGU G CUCGGUGG	794	CCACCGAG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG ACCUCGGC	2775
27	GGUGCUCG G UGGAGUCA	795	UGACUCCA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CGAGCACC	2776
32	UCGGUGGA G UCAUGGCA	796	UGCCAUGA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UCCACCGA	2777
38	GAGUCAUG G CAGUGCCC	797	GGGCACUG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CAUGACUC	2778
41	UCAUGGCA G UGCCCUUU	798	AAAGGGCA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UGCCAUGA	2779
43	AUGGCAGU G CCCUUUGU	799	ACAAAGGG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG ACUGCCAU	2780
50	UGCCCUUU G UGGAAGAC	800	GUCUUCCA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AAAGGGCA	2781
68	GGGACUUG G UGCAAACC	801	GGUUUGCA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CAAGUCCC	2782
70	GACUUGGU G CAAACCCU	802	AGGGUUUG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG ACCAAGUC	2783
87	GGGAGAAG G UGCCUAUG	803	CAUAGGCA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CUUCUCCC	2784
89	GAGAAGGU G CCUAUGGA	804	UCCAUAGG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG ACCUUCUC	2785
101	AUGGAGAA G UUCAACUU	805	AAGUUGAA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UUCUCCAU	2786
110	UUCAACUU G CUGUGAAU	806	AUUCACAG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AAGUUGAA	2787
113	AACUUGCU G UGAAUAGA	807	UCUAUUCA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AGCAAGUU	2788
122	UGAAUAGA G UAACUGAA	808	UUCAGUUA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UCUAUUCA	2789
134	CUGAAGAA G CAGUCGCA	809	UGCGACUG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UUCUUCAG	2790
137	AAGAAGCA G UCGCAGUG	810	CACUGCGA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UGCUUCUU	2791
140	AAGCAGUC G CAGUGAAG	811	CUUCACUG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG GACUGCUU	2792
143	CAGUCGCA G UGAAGAUU	812	AAUCUUCA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UGCGACUG	2793
152	UGAAGAUU G UAGAUAUG	813	CAUAUCUA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AAUCUUCA	2794
163	GAUAUGAA G CGUGCCGU	814	ACGGCACG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UUCAUAUC	2795
165	UAUGAAGC G UGCCGUAG	815	CUACGGCA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG GCUUCAUA	2796
167	UGAAGCGU G CCGUAGAC	816	GUCUACGG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG ACGCUUCA	2797
170	AGCGUGCC G UAGACUGU	817	ACAGUCUA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG GGCACGCU	2798
177	CGUAGACU G UCCAGAAA	818	UUUCUGGA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AGUCUACG	2799

204	AGAGAUCU G UAUCAAUA	819	UAUUGAUA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AGAUCUCU	2800
217	AAUAAAAU G CUAAAUCA	820	UGAUUUAG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AUUUUAUU	2801
233	AUGAAAAU G UAGUAAAA	821	UUUUACUA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AUUUUCAU	2802
236	AAAAUGUA G UAAAAUUC	822	GAAUUUUA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UACAUUUU	2803
249	AUUCUAUG G UCACAGGA	823	UCCUGUGA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CAUAGAAU	2804
264	GAGAGAAG G CAAUAUCC	824	GGAUAUUG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CUUCUCUC	2805
289	UUUCUGGA G UACUGUAG	825	CUACAGUA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UCCAGAAA	2806
294	GGAGUACU G UAGUGGAG	826	CUCCACUA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AGUACUCC	2807
297	GUACUGUA G UGGAGGAG	827	CUCCUCCA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UACAGUAC	2808
307	GGAGGAGA G CUUUUUGA	828	UCAAAAAG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UCUCCUCC	2809
325	AGAAUAGA G CCAGACAU	829	AUGUCUGG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UCUAUUCU	2810
336	AGACAUAG G CAUGCCUG	830	CAGGCAUG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CUAUGUCU	2811
340	AUAGGCAU G CCUGAACC	831	GGUUCAGG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AUGCCUAU	2812
353	AACCAGAU G CUCAGAGA	832	UCUCUGAG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AUCUGGUU	2813
380	AACUCAUG G CAGGGGUG	833	CACCCCUG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CAUGAGUU	2814
386	UGGCAGGG G UGGUUUAU	834	AUAAACCA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CCCUGCCA	2815
389	CAGGGGUG G UUUAUCUG	835	CAGAUAAA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CACCCCUG	2816
397	GUUUAUCU G CAUGGUAU	836	AUACCAUG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AGAUAAAC	2817
402	UCUGCAUG G UAUUGGAA	837	UUCCAAUA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CAUGCAGA	2818
445	AAUCUUCU G UUGGAUGA	838	UCAUCCAA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AGAAGAUU	2819
483	AGACUUUG G CUUGGCAA	839	UUGCCAAG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CAAAGUCU	2820
488	UUGGCUUG G CAACAGUA	840	UACUGUUG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CAAGCCAA	2821
494	UGGCAACA G UAUUUCGG	841	CCGAAAUA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UGUUGCCA	2822
502	GUAUUUCG G UAUAAUAA	842	UUAUUAUA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CGAAAUAC	2823
513	UAAUAAUC G UGAGCGUU	843	AACGCUCA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG GAUUAUUA	2824
517	AAUCGUGA G CGUUUGUU	844	AACAAACG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UCACGAUU	2825
519	UCGUGAGC G UUUGUUGA	845	UCAACAAA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG GCUCACGA	2826
523	GAGCGUUU G UUGAACAA	846	UUGUUCAA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AAACGCUC	2827
535	AACAAGAU G UGUGGUAC	847	GUACCACA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AUCUUGUU	2828
537	CAAGAUGU G UGGUACUU	848	AAGUACCA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG ACAUCUUG	2829
540	GAUGUGUG G UACUUUAC	849	GUAAAGUA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CACACAUC	2830

554	UACCAUAU G UUGCUCCA	850	UGGAGCAA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AUAUGGUA	2831
557	CAUAUGUU G CUCCAGAA	851	UUCUGGAG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AACAUAUG	2832
590	AAUUUCAU G CAGAACCA	852	UGGUUCUG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AUGAAAUU	2833
599	CAGAACCA G UUGAUGUU	853	AACAUCAA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UGGUUCUG	2834
605	CAGUUGAU G UUUGGUCC	854	GGACCAAA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AUCAACUG	2835
610	GAUGUUUG G UCCUGUGG	855	CCACAGGA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CAAACAUC	2836
615	UUGGUCCU G UGGAAUAG	856	CUAUUCCA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AGGACCAA	2837
623	GUGGAAUA G UACUUACU	857	AGUAAGUA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UAUUCCAC	2838
632	UACUUACU G CAAUGCUC	858	GAGCAUUG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AGUAAGUA	2839
637	ACUGCAAU G CUCGCUGG	859	CCAGCGAG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AUUGCAGU	2840
641	CAAUGCUC G CUGGAGAA	860	UUCUCCAG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG GAGCAUUG	2841
652	GGAGAAUU G CCAUGGGA	861	UCCCAUGG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AAUUCUCC	2842
669	CCAACCCA G UGACAGCU	862	AGCUGUCA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UGGGUUGG	2843
675	CAGUGACA G CUGUCAGG	863	CCUGACAG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UGUCACUG	2844
678	UGACAGCU G UCAGGAGU	864	ACUCCUGA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AGCUGUCA	2845
685	UGUCAGGA G UAUUCUGA	865	UCAGAAUA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UCCUGACA	2846
743	UCGAUUCU G CUCCUCUA	866	UAGAGGAG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AGAAUCGA	2847
752	CUCCUCUA G CUCUGCUG	867	CAGCAGAG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UAGAGGAG	2848
757	CUAGCUCU G CUGCAUAA	868	UUAUGCAG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AGAGCUAG	2849
760	GCUCUGCU G CAUAAAAU	869	AUUUUAUG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AGCAGAGC	2850
773	AAAUCUUA G UUGAGAAU	870	AUUCUCAA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UAAGAUUU	2851
788	AUCCAUCA G CAAGAAUU	871	AAUUCUUG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UGAUGGAU	2852
826	GAUAGAUG G UACAACAA	872	UUGUUGUA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CAUCUAUC	2853
851	AGAAAGGG G CAAAAAGG	873	CCUUUUUG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CCCUUUCU	2854
859	GCAAAAAG G CCCCGAGU	874	ACUCGGGG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CUUUUUGC	2855
866	GGCCCCGA G UCACUUCA	875	UGAAGUGA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UCGGGGCC	2856
876	CACUUCAG G UGGUGUGU	876	ACACACCA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CUGAAGUG	2857
879	UUCAGGUG G UGUGUCAG	877	CUGACACA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CACCUGAA	2858
881	CAGGUGGU G UGUCAGAG	878	CUCUGACA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG ACCACCUG	2859
883	GGUGGUGU G UCAGAGUC	879	GACUCUGA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG ACACCACC	2860
889	GUGUCAGA G UCUCCCAG	880	CUGGGAGA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UCUGACAC	2861

897	GUCUCCCA G UGGAUUUU	881	AAAAUCCA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UGGGAGAC	2862
910	UUUUCUAA G CACAUUCA	882	UGAAUGUG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UUAGAAAA	2863
941	UCUCUCCA G UAAACAGU	883	ACUGUUUA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UGGAGAGA	2864
948	AGUAAACA G UGCUUCUA	884	UAGAAGCA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UGUUUACU	2865
950	UAAACAGU G CUUCUAGU	885	ACUAGAAG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG ACUGUUUA	2866
957	UGCUUCUA G UGAAGAAA	886	UUUCUUCA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UAGAAGCA	2867
968	AAGAAAAU G UGAAGUAC	887	GUACUUCA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AUUUUCUU	2868
973	AAUGUGAA G UACUCCAG	888	CUGGAGUA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UUCACAUU	2869
981	GUACUCCA G UUCUCAGC	889	GCUGAGAA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UGGAGUAC	2870
988	AGUUCUCA G CCAGAACC	890	GGUUCUGG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UGAGAACU	2871
999	AGAACCCC G CACAGGUC	891	GACCUGUG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG GGGGUUCU	2872
1005	CCGCACAG G UCUUUCCU	892	AGGAAAGA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CUGUGCGG	2873
1026	GGAUACCA G CCCCUCAU	893	AUGAGGGG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UGGUAUCC	2874
1049	AUAAAUUG G UACAAGGG	894	CCCUUGUA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CAAUUUAU	2875
1062	AGGGAUCA G CUUUUCCC	895	GGGAAAAG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UGAUCCCU	2876
1072	UUUUCCCA G CCCACAUG	896	CAUGUGGG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UGGGAAAA	2877
1080	GCCCACAU G UCCUGAUC	897	GAUCAGGA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AUGUGGGC	2878
1093	GAUCAUAU G CUUUUGAA	898	UUCAAAAG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AUAUGAUC	2879
1104	UUUGAAUA G UCAGUUAC	899	GUAACUGA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UAUUCAAA	2880
1108	AAUAGUCA G UUACUUGG	900	CCAAGUAA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UGACUAUU	2881
1116	GUUACUUG G CACCCCAG	901	CUGGGGUG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CAAGUAAC	2882
1144	AACCCCUG G CAGCGGUU	902	AACCGCUG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CAGGGGUU	2883
1147	CCCUGGCA G CGGUUGGU	903	ACCAACCG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UGCCAGGG	2884
1150	UGGCAGCG G UUGGUCAA	904	UUGACCAA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CGCUGCCA	2885
1154	AGCGGUUG G UCAAAAGA	905	UCUUUUGA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CAACCGCU	2886
1190	AAUUGGAU G CAGACAAA	906	UUUGUCUG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AUCCAAUU	2887
1209	UUAUCAAU G CCUGAAAG	907	CUUUCAGG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AUUGAUAA	2888
1224	AGAGACUU G UGAGAAGU	908	ACUUCUCA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AAGUCUCU	2889
1231	UGUGAGAA G UUGGGCUA	909	UAGCCCAA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UUCUCACA	2890
1236	GAAGUUGG G CUAUCAAU	910	AUUGAUAG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CCAACUUC	2891
1254	GAAGAAAA G UUGUAUGA	911	UCAUACAA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UUUUCUUC	2892

1257	GAAAAGUU G UAUGAAUC	912	GAUUCAUA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AACUUUUC	2893
1268	UGAAUCAG G UUACUAUA	913	UAUAGUAA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CUGAUUCA	2894
1316	UUUUCAAA G UGAAUUUG	914	CAAAUUCA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UUUGAAAA	2895
1324	GUGAAUUU G UUAGAAAU	915	AUUUCUAA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AAAUUCAC	2896
1349	AAAUAUUG G UUGACUUC	916	GAAGUCAA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CAAUAUUU	2897
1360	GACUUCCG G CUUUCUAA	917	UUAGAAAG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CGGAAGUC	2898
1371	UUCUAAGG G UGAUGGAU	918	AUCCAUCA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CCUUAGAA	2899
1384	GGAUUGGA G UUCAAGAG	919	CUCUUGAA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UCCAAUCC	2900
1417	AAAGGGAA G CUGAUUGA	920	UCAAUCAG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UUCCCUUU	2901
1430	UUGAUAUU G UGAGCAGC	921	GCUGCUCA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AAUAUCAA	2902
1434	UAUUGUGA G CAGCCAGA	922	UCUGGCUG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UCACAAUA	2903
1437	UGUGAGCA G CCAGAAGG	923	CCUUCUGG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UGCUCACA	2904
1445	GCCAGAAG G UUUGGCUU	924	AAGCCAAA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CUUCUGGC	2905
1450	AAGGUUUG G CUUCCUGC	925	GCAGGAAG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CAAACCUU	2906
1457	GGCUUCCU G CCACAUGA	926	UCAUGUGG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AGGAAGCC	2907
1477	GACCAUCG G CUCUGGGG	927	CCCCAGAG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CGAUGGUC	2908
1493	GAAUCCUG G UGAAUAUA	928	UAUAUUCA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CAGGAUUC	2909
1502	UGAAUAUA G UGCUGCUA	929	UAGCAGCA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UAUAUUCA	2910
1504	AAUAUAGU G CUGCUAUG	930	CAUAGCAG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG ACUAUAUU	2911
1507	AUAGUGCU G CUAUGUUG	931	CAACAUAG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AGCACUAU	2912
1512	GCUGCUAU G UUGACAUU	932	AAUGUCAA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AUAGCAGC	2913
1545	AUUAUCCU G UCCUGCAA	933	UUGCAGGA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AGGAUAAU	2914
1550	CCUGUCCU G CAAACUGC	934	GCAGUUUG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AGGACAGG	2915
1557	UGCAAACU G CAAAUAGU	935	ACUAUUUG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AGUUUGCA	2916
1564	UGCAAAUA G UAGUUCCU	936	AGGAACUA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UAUUUGCA	2917
1567	AAAUAGUA G UUCCUGAA	937	UUCAGGAA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UACUAUUU	2918
1576	UUCCUGAA G UGUUCACU	938	AGUGAACA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UUCAGGAA	2919
1578	CCUGAAGU G UUCACUUC	939	GAAGUGAA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG ACUUCAGG	2920
1590	ACUUCCCU G UUUAUCCA	940	UGGAUAAA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AGGGAAGU	2921
1619	UUUAUUUU G UUUGUUCG	941	CGAACAAA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AAAAUAAA	2922
1623	UUUUGUUU G UUCGGCAU	942	AUGCCGAA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AAACAAAA	2923

1628	UUUGUUCG G CAUACAAA	943	UUUGUAUG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CGAACAAA	2924
1656	UCUUAAUU G UAAGCAAA	944	UUUGCUUA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AAUUAAGA	2925
1660	AAUUGUAA G CAAAACUU	945	AAGUUUUG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UUACAAUU	2926
1710	UUCUUCAU G UGUGUUUA	946	UAAACACA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AUGAAGAA	2927
1712	CUUCAUGU G UGUUUAGU	947	ACUAAACA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG ACAUGAAG	2928
1714	UCAUGUGU G UUUAGUAU	948	AUACUAAA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG ACACAUGA	2929
1719	UGUGUUUA G UAUCUGAA	949	UUCAGAUA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UAAACACA	2930
1743	CUCAUCUG G UGGAAACC	950	GGUUUCCA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CAGAUGAG	2931
1754	GAAACCAA G UUUCAGGG	951	CCCUGAAA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UUGGUUUC	2932
1770	GGACAUGA G UUUUCCAG	952	CUGGAAAA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UCAUGUCC	2933
1778	GUUUUCCA G CUUUUAUA	953	UAUAAAAG GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UGGAAAAC	2934
1792	AUACACAC G UAUCUCAU	954	AUGAGAUA GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG GUGUGUAU	2935
17	AGUCCGCC G AGGUGCUC	1186	GAGCACCU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG GGCGGACU	2936
19	UCCGCCGA G GUGCUCGG	1187	CCGAGCAC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UCGGCGGA	2937
26	AGGUGCUC G GUGGAGUC	1188	GACUCCAC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG GAGCACCU	2938
29	UGCUCGGU G GAGUCAUG	1189	CAUGACUC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG ACCGAGCA	2939
30	GCUCGGUG G AGUCAUGG	1190	CCAUGACU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CACCGAGC	2940
37	GGAGUCAU G GCAGUGCC	1191	GGCACUGC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AUGACUCC	2941
52	CCCUUUGU G GAAGACUG	1192	CAGUCUUC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG ACAAAGGG	2942
53	CCUUUGUG G AAGACUGG	1193	CCAGUCUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CACAAAGG	2943
56	UUGUGGAA G ACUGGGAC	1194	GUCCCAGU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UUCCACAA	2944
60	GGAAGACU G GGACUUGG	1195	CCAAGUCC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AGUCUUCC	2945
61	GAAGACUG G GACUUGGU	1196	ACCAAGUC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CAGUCUUC	2946
62	AAGACUGG G ACUUGGUG	1197	CACCAAGU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CCAGUCUU	2947
67	UGGGACUU G GUGCAAAC	1198	GUUUGCAC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AAGUCCCA	2948
79	CAAACCCU G GGAGAAGG	1199	CCUUCUCC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AGGGUUUG	2949
80	AAACCCUG G GAGAAGGU	1200	ACCUUCUC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CAGGGUUU	2950
81	AACCCUGG G AGAAGGUG	1201	CACCUUCU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CCAGGGUU	2951
83	CCCUGGGA G AAGGUGCC	1202	GGCACCUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UCCCAGGG	2952
86	UGGGAGAA G GUGCCUAU	1203	AUAGGCAC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UUCUCCCA	2953
95	GUGCCUAU G GAGAAGUU	1204	AACUUCUC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AUAGGCAC	2954

96	UGCCUAUG G AGAAGUUC	1205	GAACUUCU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CAUAGGCA	2955
98	CCUAUGGA G AAGUUCAA	1206	UUGAACUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UCCAUAGG	2956
115	CUUGCUGU G AAUAGAGU	1207	ACUCUAUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG ACAGCAAG	2957
120	UGUGAAUA G AGUAACUG	1208	CAGUUACU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UAUUCACA	2958
128	GAGUAACU G AAGAAGCA	1209	UGCUUCUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AGUUACUC	2959
131	UAACUGAA G AAGCAGUC	1210	GACUGCUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UUCAGUUA	2960
145	GUCGCAGU G AAGAUUGU	1211	ACAAUCUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG ACUGCGAC	2961
148	GCAGUGAA G AUUGUAGA	1212	UCUACAAU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UUCACUGC	2962
155	AGAUUGUA G AUAUGAAG	1213	CUUCAUAU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UACAAUCU	2963
160	GUAGAUAU G AAGCGUGC	1214	GCACGCUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AUAUCUAC	2964
173	GUGCCGUA G ACUGUCCA	1215	UGGACAGU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UACGGCAC	2965
182	ACUGUCCA G AAAAUAUU	1216	AAUAUUUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UGGACAGU	2966
193	AAUAUUAA G AAAGAGAU	1217	AUCUCUUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UUAAUAUU	2967
197	UUAAGAAA G AGAUCUGU	1218	ACAGAUCU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UUUCUUAA	2968
199	AAGAAAGA G AUCUGUAU	1219	AUACAGAU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UCUUUCUU	2969
227	UAAAUCAU G AAAAUGUA	1220	UACAUUUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AUGAUUUA	2970
248	AAUUCUAU G GUCACAGG	1221	CCUGUGAC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AUAGAAUU	2971
255	UGGUCACA G GAGAGAAG	1222	CUUCUCUC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UGUGACCA	2972
256	GGUCACAG G AGAGAAGG	1223	CCUUCUCU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CUGUGACC	2973
258	UCACAGGA G AGAAGGCA	1224	UGCCUUCU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UCCUGUGA	2974
260	ACAGGAGA G AAGGCAAU	1225	AUUGCCUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UCUCCUGU	2975
263	GGAGAGAA G GCAAUAUC	1226	GAUAUUGC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UUCUCUCC	2976
286	UUAUUUCU G GAGUACUG	1227	CAGUACUC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AGAAAUAA	2977
287	UAUUUCUG G AGUACUGU	1228	ACAGUACU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CAGAAAUA	2978
299	ACUGUAGU G GAGGAGAG	1229	CUCUCCUC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG ACUACAGU	2979
300	CUGUAGUG G AGGAGAGC	1230	GCUCUCCU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CACUACAG	2980
302	GUAGUGGA G GAGAGCUU	1231	AAGCUCUC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UCCACUAC	2981
303	UAGUGGAG G AGAGCUUU	1232	AAAGCUCU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CUCCACUA	2982
305	GUGGAGGA G AGCUUUUU	1233	AAAAAGCU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UCCUCCAC	2983
314	AGCUUUUU G ACAGAAUA	1234	UAUUCUGU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AAAAAGCU	2984
318	UUUUGACA G AAUAGAGC	1235	GCUCUAUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UGUCAAAA	2985

323	ACAGAAUA G AGCCAGAC	1236	GUCUGGCU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UAUUCUGU	2986
329	UAGAGCCA G ACAUAGGC	1237	GCCUAUGU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UGGCUCUA	2987
335	CAGACAUA G GCAUGCCU	1238	AGGCAUGC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UAUGUCUG	2988
344	GCAUGCCU G AACCAGAU	1239	AUCUGGUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AGGCAUGC	2989
350	CUGAACCA G AUGCUCAG	1240	CUGAGCAU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UGGUUCAG	2990
358	GAUGCUCA G AGAUUCUU	1241	AAGAAUCU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UGAGCAUC	2991
360	UGCUCAGA G AUUCUUCC	1242	GGAAGAAU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UCUGAGCA	2992
379	CAACUCAU G GCAGGGGU	1243	ACCCCUGC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AUGAGUUG	2993
383	UCAUGGCA G GGGUGGUU	1244	AACCACCC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UGCCAUGA	2994
384	CAUGGCAG G GGUGGUUU	1245	AAACCACC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CUGCCAUG	2995
385	AUGGCAGG G GUGGUUUA	1246	UAAACCAC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CCUGCCAU	2996
388	GCAGGGGU G GUUUAUCU	1247	AGAUAAAC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG ACCCCUGC	2997
401	AUCUGCAU G GUAUUGGA	1248	UCCAAUAC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AUGCAGAU	2998
407	AUGGUAUU G GAAUAACU	1249	AGUUAUUC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AAUACCAU	2999
408	UGGUAUUG G AAUAACUC	1250	GAGUUAUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CAAUACCA	3000
420	AACUCACA G GGAUAUUA	1251	UAAUAUCC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UGUGAGUU	3001
421	ACUCACAG G GAUAUUAA	1252	UUAAUAUC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CUGUGAGU	3002
422	CUCACAGG G AUAUUAAA	1253	UUUAAUAU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CCUGUGAG	3003
434	UUAAACCA G AAAAUCUU	1254	AAGAUUUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UGGUUUAA	3004
448	CUUCUGUU G GAUGAAAG	1255	CUUUCAUC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AACAGAAG	3005
449	UUCUGUUG G AUGAAAGG	1256	CCUUUCAU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CAACAGAA	3006
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456	GGAUGAAA G GGAUAACC	1258	GGUUAUCC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UUUCAUCC	3008
457	GAUGAAAG G GAUAACCU	1259	AGGUUAUC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CUUUCAUC	3009
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501	AGUAUUUC G GUAUAAUA	1264	UAUUAUAC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG GAAAUACU	3014
515	AUAAUCGU G AGCGUUUG	1265	CAAACGCU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG ACGAUUAU	3015
526	CGUUUGUU G AACAAGAU	1266	AUCUUGUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AACAAACG	3016

532	UUGAACAA G AUGUGUGG	1267	CCACACAU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UUGUUCAA	3017
539	AGAUGUGU G GUACUUUA	1268	UAAAGUAC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG ACACAUCU	3018
563	UUGCUCCA G AACUUCUG	1269	CAGAAGUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UGGAGCAA	3019
571	GAACUUCU G AAGAGAAG	1270	CUUCUCUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AGAAGUUC	3020
574	CUUCUGAA G AGAAGAGA	1271	UCUCUUCU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UUCAGAAG	3021
576	UCUGAAGA G AAGAGAAU	1272	AUUCUCUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UCUUCAGA	3022
579	GAAGAGAA G AGAAUUUC	1273	GAAAUUCU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UUCUCUUC	3023
581	AGAGAAGA G AAUUUCAU	1274	AUGAAAUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UCUUCUCU	3024
593	UUCAUGCA G AACCAGUU	1275	AACUGGUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UGCAUGAA	3025
602	AACCAGUU G AUGUUUGG	1276	CCAAACAU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AACUGGUU	3026
609	UGAUGUUU G GUCCUGUG	1277	CACAGGAC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AAACAUCA	3027
617	GGUCCUGU G GAAUAGUA	1278	UACUAUUC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG ACAGGACC	3028
618	GUCCUGUG G AAUAGUAC	1279	GUACUAUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CACAGGAC	3029
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645	GCUCGCUG G AGAAUUGC	1281	GCAAUUCU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CAGCGAGC	3031
647	UCGCUGGA G AAUUGCCA	1282	UGGCAAUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UCCAGCGA	3032
657	AUUGCCAU G GGACCAAC	1283	GUUGGUCC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AUGGCAAU	3033
658	UUGCCAUG G GACCAACC	1284	GGUUGGUC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CAUGGCAA	3034
659	UGCCAUGG G ACCAACCC	1285	GGGUUGGU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CCAUGGCA	3035
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696	UUCUGACU G GAAAGAAA	1290	UUUCUUUC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AGUCAGAA	3040
697	UCUGACUG G AAAGAAAA	1291	UUUUCUUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CAGUCAGA	3041
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726	CAACCCUU G GAAAAAAA	1293	UUUUUUUC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AAGGGUUG	3043
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818	UCAAAAAA G AUAGAUGG	1300	CCAUCUAU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UUUUUUGA	3050
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848	UCAAGAAA G GGGCAAAA	1304	UUUUGCCC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UUUCUUGA	3054
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858	GGCAAAAA G GCCCCGAG	1307	CUCGGGGC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UUUUUGCC	3057
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1143	GAACCCCU G GCAGCGGU	1335	ACCGCUGC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AGGGGUUC	3085
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1153	CAGCGGUU G GUCAAAAG	1337	CUUUUGAC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AACCGCUG	3087
1161	GGUCAAAA G AAUGACAC	1338	GUGUCAUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UUUUGACC	3088
1165	AAAAGAAU G ACACGAUU	1339	AAUCGUGU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AUUCUUUU	3089
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1187	CCAAAUUG G AUGCAGAC	1342	GUCUGCAU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CAAUUUGG	3092
1193	UGGAUGCA G ACAAAUCU	1343	AGAUUUGU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UGCAUCCA	3093
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1219	CUGAAAGA G ACUUGUGA	1346	UCACAAGU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UCUUUCAG	3096
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1246	UAUCAAUG G AAGAAAAG	1352	CUUUUCUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CAUUGAUA	3102
1249	CAAUGGAA G AAAAGUUG	1353	CAACUUUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UUCCAUUG	3103
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1267	AUGAAUCA G GUUACUAU	1355	AUAGUAAC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UGAUUCAU	3105
1286	CAACAACU G AUAGGAGA	1356	UCUCCUAU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AGUUGUUG	3106
1290	AACUGAUA G GAGAAACA	1357	UGUUUCUC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UAUCAGUU	3107
1291	ACUGAUAG G AGAAACAA	1358	UUGUUUCU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CUAUCAGU	3108
1293	UGAUAGGA G AAACAAUA	1359	UAUUGUUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UCCUAUCA	3109

1318	UUCAAAGU G AAUUUGUU	1360	AACAAAUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG ACUUUGAA	3110
1328	AUUUGUUA G AAAUGGAU	1361	AUCCAUUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UAACAAAU	3111
1333	UUAGAAAU G GAUGAUAA	1362	UUAUCAUC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AUUUCUAA	3112
1334	UAGAAAUG G AUGAUAAA	1363	UUUAUCAU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CAUUUCUA	3113
1337	AAAUGGAU G AUAAAAUA	1364	UAUUUUAU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AUCCAUUU	3114
1348	AAAAUAUU G GUUGACUU	1365	AAGUCAAC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AAUAUUUU	3115
1352	UAUUGGUU G ACUUCCGG	1366	CCGGAAGU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AACCAAUA	3116
1359	UGACUUCC G GCUUUCUA	1367	UAGAAAGC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG GGAAGUCA	3117
1369	CUUUCUAA G GGUGAUGG	1368	CCAUCACC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UUAGAAAG	3118
1370	UUUCUAAG G GUGAUGGA	1369	UCCAUCAC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CUUAGAAA	3119
1373	CUAAGGGU G AUGGAUUG	1370	CAAUCCAU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG ACCCUUAG	3120
1376	AGGGUGAU G GAUUGGAG	1371	CUCCAAUC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AUCACCCU	3121
1377	GGGUGAUG G AUUGGAGU	1372	ACUCCAAU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CAUCACCC	3122
1381	GAUGGAUU G GAGUUCAA	1373	UUGAACUC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AAUCCAUC	3123
1382	AUGGAUUG G AGUUCAAG	1374	CUUGAACU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CAAUCCAU	3124
1390	GAGUUCAA G AGACACUU	1375	AAGUGUCU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UUGAACUC	3125
1392	GUUCAAGA G ACACUUCC	1376	GGAAGUGU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UCUUGAAC	3126
1402	CACUUCCU G AAGAUUAA	1377	UUAAUCUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AGGAAGUG	3127
1405	UUCCUGAA G AUUAAAGG	1378	CCUUUAAU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UUCAGGAA	3128
1412	AGAUUAAA G GGAAGCUG	1379	CAGCUUCC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UUUAAUCU	3129
1413	GAUUAAAG G GAAGCUGA	1380	UCAGCUUC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CUUUAAUC	3130
1414	AUUAAAGG G AAGCUGAU	1381	AUCAGCUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CCUUUAAU	3131
1420	GGGAAGCU G AUUGAUAU	1382	AUAUCAAU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AGCUUCCC	3132
1424	AGCUGAUU G AUAUUGUG	1383	CACAAUAU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AAUCAGCU	3133
1432	GAUAUUGU G AGCAGCCA	1384	UGGCUGCU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG ACAAUAUC	3134
1441	AGCAGCCA G AAGGUUUG	1385	CAAACCUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UGGCUGCU	3135
1444	AGCCAGAA G GUUUGGCU	1386	AGCCAAAC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UUCUGGCU	3136
1449	GAAGGUUU G GCUUCCUG	1387	CAGGAAGC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AAACCUUC	3137
1464	UGCCACAU G AUCGGACC	1388	GGUCCGAU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AUGUGGCA	3138
1468	ACAUGAUC G GACCAUCG	1389	CGAUGGUC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG GAUCAUGU	3139
1469	CAUGAUCG G ACCAUCGG	1390	CCGAUGGU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CGAUCAUG	3140

1476	GGACCAUC G GCUCUGGG	1391	CCCAGAGC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG GAUGGUCC	3141
1482	UCGGCUCU G GGGAAUCC	1392	GGAUUCCC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AGAGCCGA	3142
1483	CGGCUCUG G GGAAUCCU	1393	AGGAUUCC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CAGAGCCG	3143
1484	GGCUCUGG G GAAUCCUG	1394	CAGGAUUC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CCAGAGCC	3144
1485	GCUCUGGG G AAUCCUGG	1395	CCAGGAUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CCCAGAGC	3145
1492	GGAAUCCU G GUGAAUAU	1396	AUAUUCAC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AGGAUUCC	3146
1495	AUCCUGGU G AAUAUAGU	1397	ACUAUAUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG ACCAGGAU	3147
1515	GCUAUGUU G ACAUUAUU	1398	AAUAAUGU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AACAUAGC	3148
1531	UCUUCCUA G AGAAGAUU	1399	AAUCUUCU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UAGGAAGA	3149
1533	UUCCUAGA G AAGAUUAU	1400	AUAAUCUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UCUAGGAA	3150
1536	CUAGAGAA G AUUAUCCU	1401	AGGAUAAU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UUCUCUAG	3151
1573	UAGUUCCU G AAGUGUUC	1402	GAACACUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AGGAACUA	3152
1627	GUUUGUUC G GCAUACAA	1403	UUGUAUGC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG GAACAAAC	3153
1670	AAAACUUU G GGGAAAGG	1404	CCUUUCCC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AAAGUUUU	3154
1671	AAACUUUG G GGAAAGGA	1405	UCCUUUCC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CAAAGUUU	3155
1672	AACUUUGG G GAAAGGAU	1406	AUCCUUUC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CCAAAGUU	3156
1673	ACUUUGGG G AAAGGAUG	1407	CAUCCUUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CCCAAAGU	3157
1677	UGGGGAAA G GAUGAAUA	1408	UAUUCAUC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UUUCCCCA	3158
1678	GGGGAAAG G AUGAAUAG	1409	CUAUUCAU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CUUUCCCC	3159
1681	GAAAGGAU G AAUAGAAU	1410	AUUCUAUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AUCCUUUC	3160
1686	GAUGAAUA G AAUUCAUU	1411	AAUGAAUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UAUUCAUC	3161
1696	AUUCAUUU G AUUAUUUC	1412	GAAAUAAU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AAAUGAAU	3162
1725	UAGUAUCU G AAUUUGAA	1413	UUCAAAUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AGAUACUA	3163
1731	CUGAAUUU G AAACUCAU	1414	AUGAGUUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AAAUUCAG	3164
1742	ACUCAUCU G GUGGAAAC	1415	GUUUCCAC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AGAUGAGU	3165
1745	CAUCUGGU G GAAACCAA	1416	UUGGUUUC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG ACCAGAUG	3166
1746	AUCUGGUG G AAACCAAG	1417	CUUGGUUU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CACCAGAU	3167
1760	AAGUUUCA G GGGACAUG	1418	CAUGUCCC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG UGAAACUU	3168
1761	AGUUUCAG G GGACAUGA	1419	UCAUGUCC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CUGAAACU	3169
1762	GUUUCAGG G GACAUGAG	1420	CUCAUGUC GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CCUGAAAC	3170
1763	UUUCAGGG G ACAUGAGU	1421	ACUCAUGU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG CCCUGAAA	3171

1768	GGGGACAU G AGUUUUCC	1422	GGAAAACU GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG AUGUCCCC	3172

Input Sequence = AF016582. Cut Site = G/.
Stem Length = 8. Core Sequence = GGAGGAAACUCC CU UCAAGGACAUCGUCCGGG
AF016582 (Homo sapiens checkpoint kinase Chk1 (CHK1) mRNA; 1821 bp)

CLAIMS

What is claimed is:

1. A nucleic acid molecule which down regulates expression of a Chk1 gene.

2. The nucleic acid of claim 1, wherein said nucleic acid molecule is used to treat cancer.

3. The nucleic acid molecule of claim 1, wherein said nucleic acid molecule is an enzymatic nucleic acid molecule.

4. The nucleic acid of claim 3, wherein a binding arm of said enzymatic nucleic acid molecule comprise sequences complementary to any of sequences defined as Sequence ID Nos. 1-1422.

5. The nucleic acid molecule of claim 3, wherein said enzymatic nucleic acid molecule comprises any of sequences defined as sequence ID Nos. 1423-3172.

6. The nucleic acid molecule of claim 1, wherein said nucleic acid molecule is an antisense nucleic acid molecule.

7. The nucleic acid molecule of claim 6, wherein said antisense nucleic acid molecule comprises sequence complementary to any of sequence defined as Sequence ID Nos. 1-1422 and 3173-3180.

8. The nucleic acid molecule of claim 6, wherein said antisense nucleic acid molecule comprise any of sequences defined as sequence ID Nos. 3181-3188.

9. The nucleic acid molecule of claim 3, wherein said enzymatic nucleic acid molecule is in a hammerhead (HH) motif.

10. The nucleic acid molecule of claim 3, wherein said enzymatic nucleic acid molecule is in a hairpin, hepatitis Delta virus, group I intron, VS nucleic acid, amberzyme, zinzyme or RNAse P nucleic acid motif.

11. The nucleic acid molecule of claim 3, wherein said enzymatic nucleic acid molecule is in a NCH motif.

12. The nucleic acid molecule of claim 3, wherein said enzymatic nucleic acid molecule is in a G-cleaver motif.

13. The nucleic acid molecule of claim 3, wherein said enzymatic nucleic acid molecule is a DNAzyme.

14. The nucleic acid molecule of claim 3, wherein said enzymatic nucleic acid molecule comprises between 12 and 100 bases complementary to the RNA of Chk1 gene.

15. The nucleic acid of claim 3, wherein said enzymatic nucleic acid molecule comprises between 14 and 24 bases complementary to the RNA of Chk1 gene.

16. The nucleic acid molecule of claim 1, wherein said nucleic acid is chemically synthesized.

17. The nucleic acid molecule of claim 1, wherein said nucleic acid comprises at least one 2'-sugar modification.

18. The nucleic acid molecule of claim 1, wherein said nucleic acid comprises at least one nucleic acid base modification.

19. The nucleic acid molecule of claim 1, wherein said nucleic acid comprises at least one phosphate backbone modification.

20. A mammalian cell including the nucleic acid molecule of claim 1.

21. The mammalian cell of claim 20, wherein said mammalian cell is a human cell.

22. A method of reducing Chk1 activity in a cell, comprising the step of contacting said cell with the nucleic acid molecule of claim 1, under conditions suitable for said reduction of Chk1 activity.

23. A method of treatment of a patient having a condition associated with the level of Chk1, comprising contacting cells of said patient with the nucleic acid molecule of claim 1, under conditions suitable for said treatment.

24. The method of claim 23 further comprising the use of one or more therapies under conditions suitable for said treatment.

25. A method of cleaving RNA of Chk1 gene, comprising, contacting the nucleic acid molecule of claim 1, with said RNA under conditions suitable for the cleavage of said RNA.

26. The method of claim 25, wherein said cleavage is carried out in the presence of a divalent cation.

27. The method of claim 26, wherein said divalent cation is Mg^{2+}.

28. The nucleic acid molecule of claim 1, wherein said nucleic acid comprises a cap structure, wherein the cap structure is at the 5'-end or 3'-end or both the 5'-end and the 3'-end.

29. The enzymatic nucleic acid molecule of claim 9, wherein said hammerhead motif comprises sequences complementary to any of sequences shown as Seq ID Nos 1-358.

30. The enzymatic nucleic acid molecule of claim 11, wherein said NCH motif comprises sequences complementary to any of sequences shown as Seq ID Nos 359-680.

31. The enzymatic nucleic acid molecule of claim 12, wherein said G-cleaver motif comprises sequences complementary to any of sequences shown as Seq ID Nos 681-790.

32. The enzymatic nucleic acid molecule of claim 13, wherein said DNAzyme comprises sequences complementary to any of substrate sequences shown as Seq. ID Nos 791-1185.

33. The enzymatic nucleic acid molecule of claim 10, wherein said zinzyme comprises sequences complementary to any of substrate sequences shown as Seq. ID Nos 791-954.

34. The enzymatic nucleic acid molecule of claim 10, wherein said amberzyme comprises sequences complementary to any of substrate sequences shown as Seq. ID Nos 791-1422.

35. An expression vector comprising nucleic acid sequence encoding at least one nucleic acid molecule of claim 1, in a manner which allows expression of that nucleic acid molecule.

36. A mammalian cell including an expression vector of claim 35.

37. The mammalian cell of claim 36, wherein said mammalian cell is a human cell.

38. The expression vector of claim 35, wherein said nucleic acid molecule is an enzymatic nucleic acid molecule.

39. The expression vector of claim 35, wherein said expression vector further comprises a sequence for an antisense nucleic acid molecule complementary to the RNA of Chk1 gene.

40. The expression vector of claim 35, wherein said expression vector comprises sequence encoding at least two said nucleic acid molecules, which may be same or different.

41. The expression vector of claim 40, wherein one said expression vector further comprises sequence encoding antisense nucleic acid molecule complementary to the RNA of Chk1 gene.

42. The expression vector of claim 40, wherein one said expression vector further comprises sequence encoding enzymatic nucleic acid molecule complementary to the RNA of Chk1 gene.

43. A method for treatment of cancer comprising the step of administering to a patient the nucleic acid molecule of claim 1 under conditions suitable for said treatment.

44. The method of claim 43, wherein said cancer is colorectal cancer.

45. The method of claim 43, wherein said cancer is lung cancer.

46. The method of claim 43, wherein said cancer is breast cancer.

47. The method of claim 43, wherein said cancer is prostate cancer.

48. A method for treatment of cancer comprising the step of administering to a patient the antisense nucleic acid molecule of claim 7 under conditions suitable for said treatment.

49. The method of claim 45, wherein said method further comprises administering to said patient the nucleic acid molecule of claim 1 in conjunction with one or more of other therapies.

50. The method of claim 49, wherein said "other therapies" are therapies selected from the group consisting of radiation and chemotherapy treatment.

51. The nucleic acid molecule of claim 7, wherein said nucleic acid molecule comprises at least five ribose residues; at least ten 2'-*O*-methyl modifications, and a 3'- end modification.

52. The nucleic acid molecule of claim 51, wherein said nucleic acid molecule further comprises phosphorothioate linkages on at least three of the 5' terminal nucleotides.

53. The nucleic acid molecule of claim 51, wherein said 3'- end modification is 3'-3' inverted abasic moiety.

54. The nucleic acid molecule of claim 3, wherein said nucleic acid molecule comprises at least five ribose residues; at least ten 2'-*O*-methyl modifications, and a 3'- end modification.

55. The nucleic acid molecule of claim 54, wherein said nucleic acid molecule further comprises phosphorothioate linkages on at least three of the 5' terminal nucleotides.

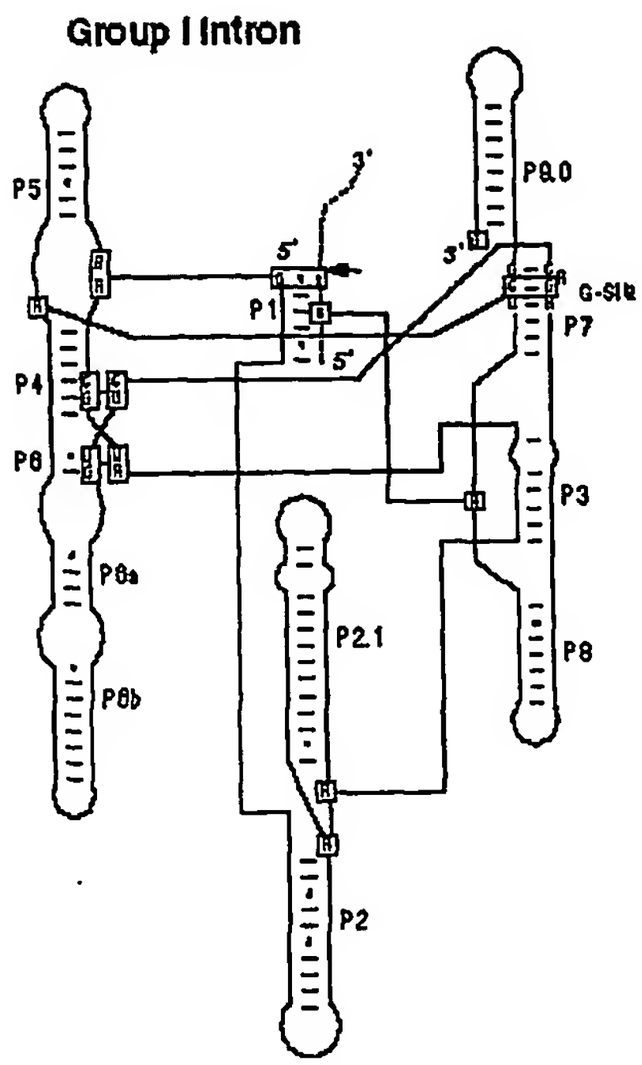
56. The nucleic acid molecule of claim 54, wherein said 3'- end modification is 3'-3' inverted abasic moiety.

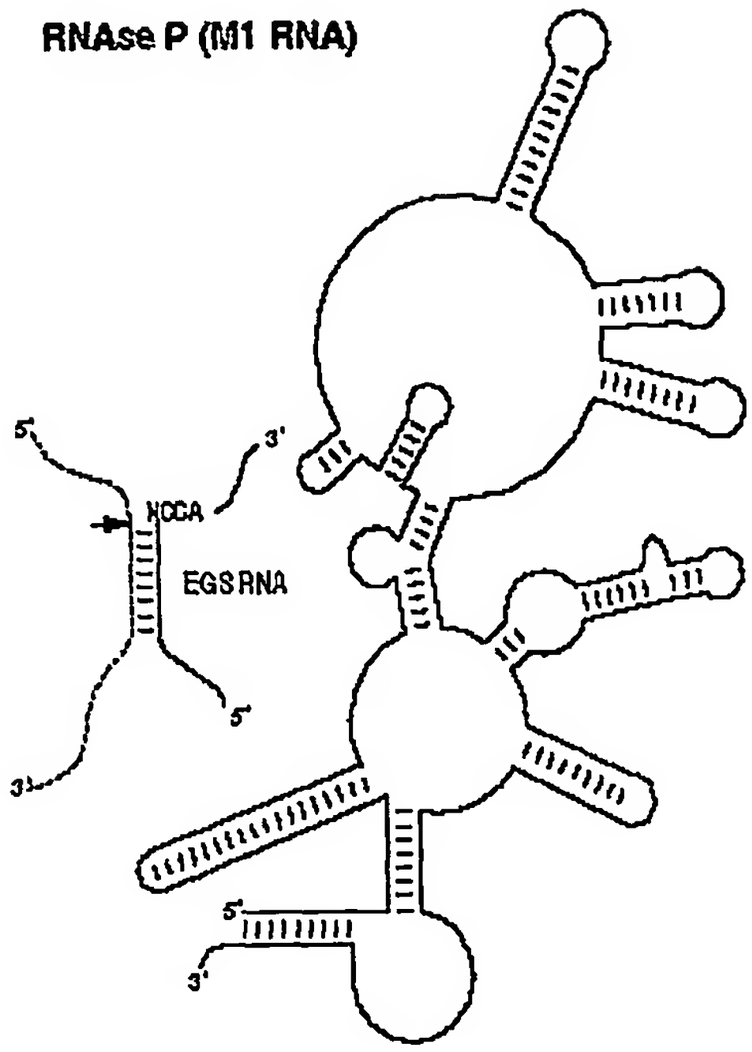
57. The enzymatic nucleic acid molecule of claim 13, wherein said DNAzyme comprises at least ten 2'-*O*-methyl modifications and a 3'-end modification.

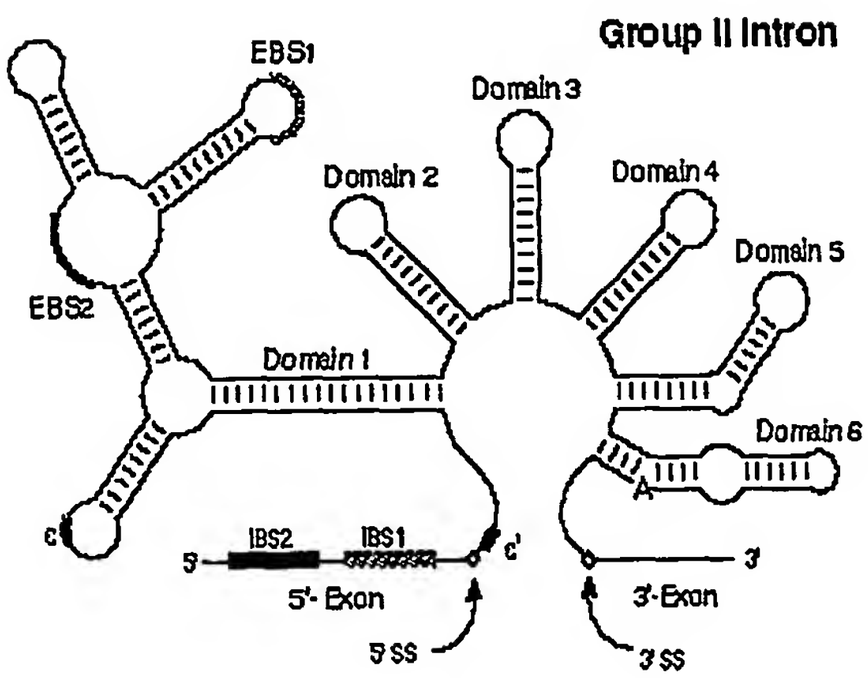
58. The enzymatic nucleic acid molecule of claim 57, wherein said DNAzyme further comprises phosphorothioate linkages on at least three of the 5' terminal nucleotides.

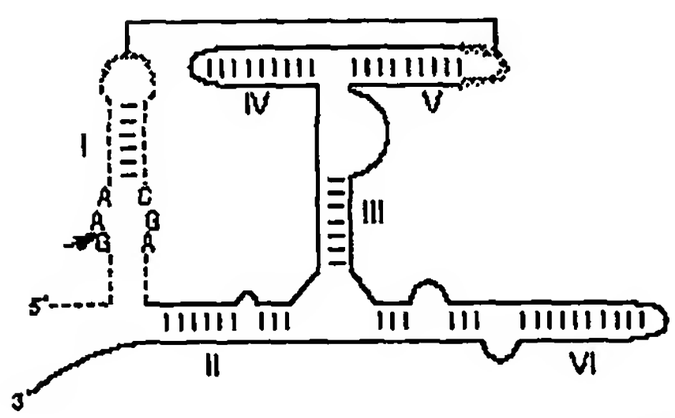
59. The enzymatic nucleic acid molecule of claim 57, wherein said 3'- end modification is 3'-3' inverted abasic moiety.

Figure 1: Ribozyme Motifs



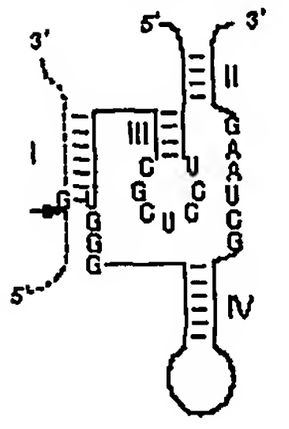


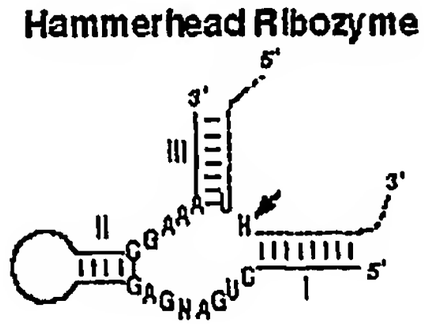


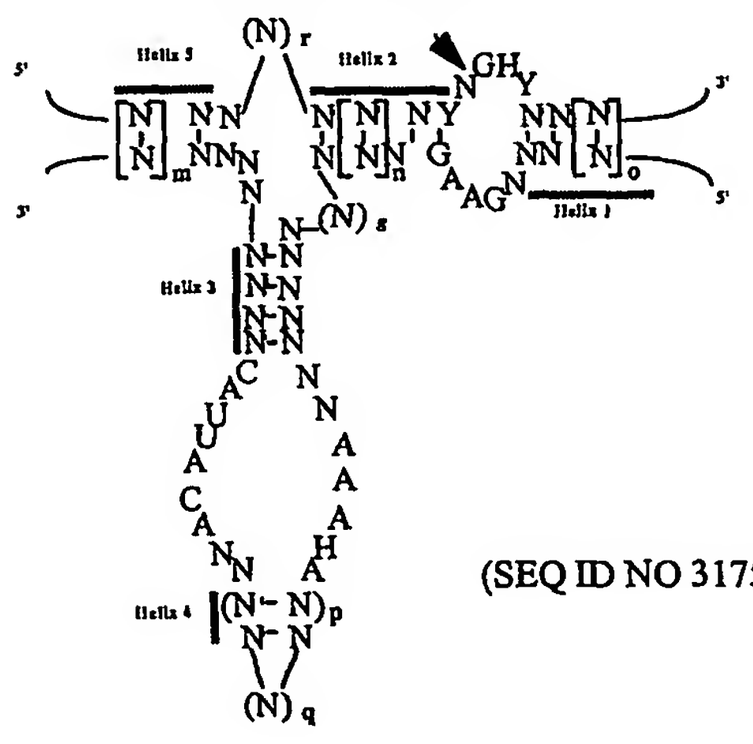


(SEQ ID NO 3174)

Hairpin Ribozyme







(SEQ ID NO 3175)

Figure 2: Examples of Nuclease Stable Ribozyme Motifs

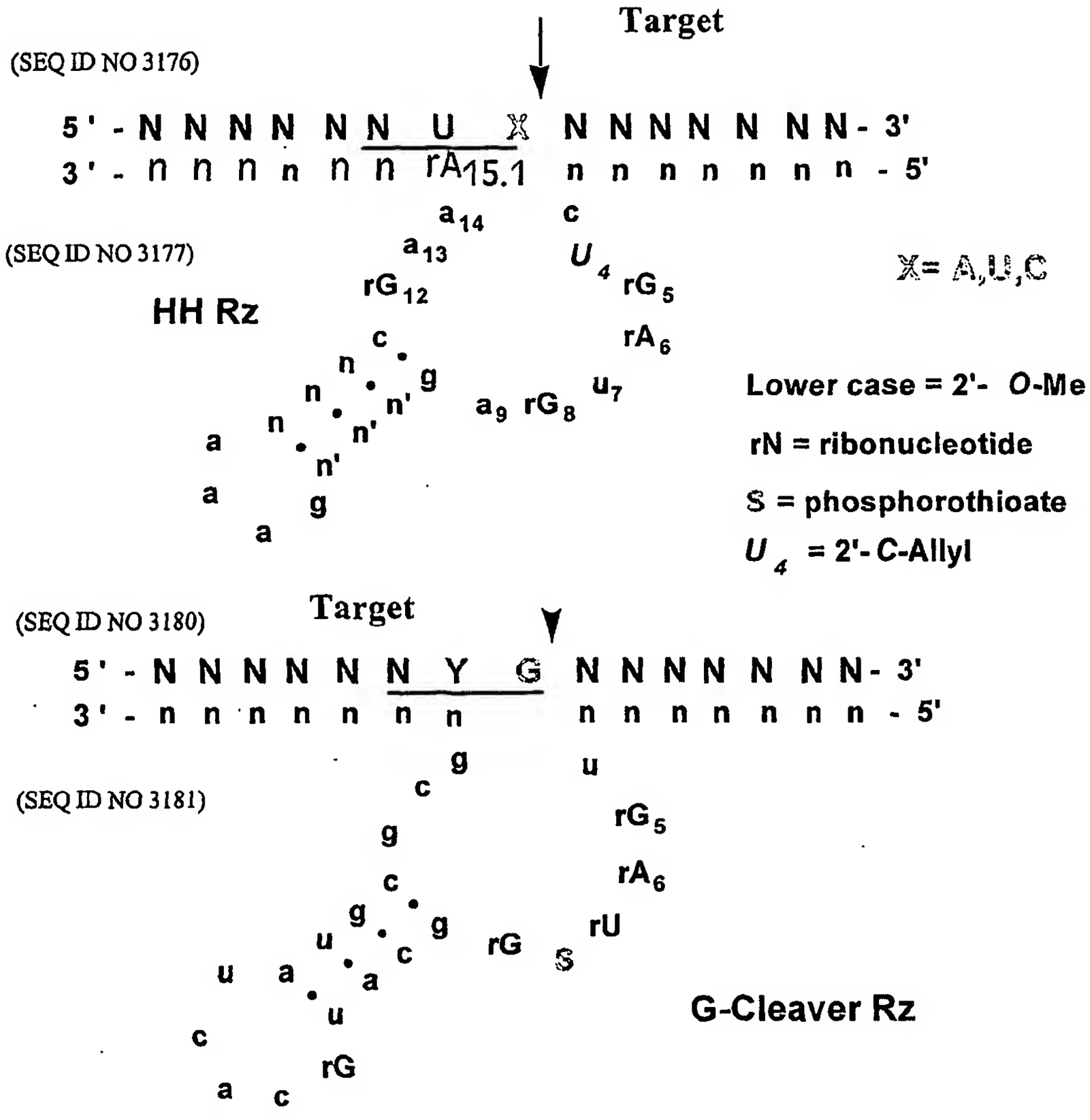


Figure 3: 2'-*O*-Me substituted Amberzyme Enzymatic Nucleic Acid Motif

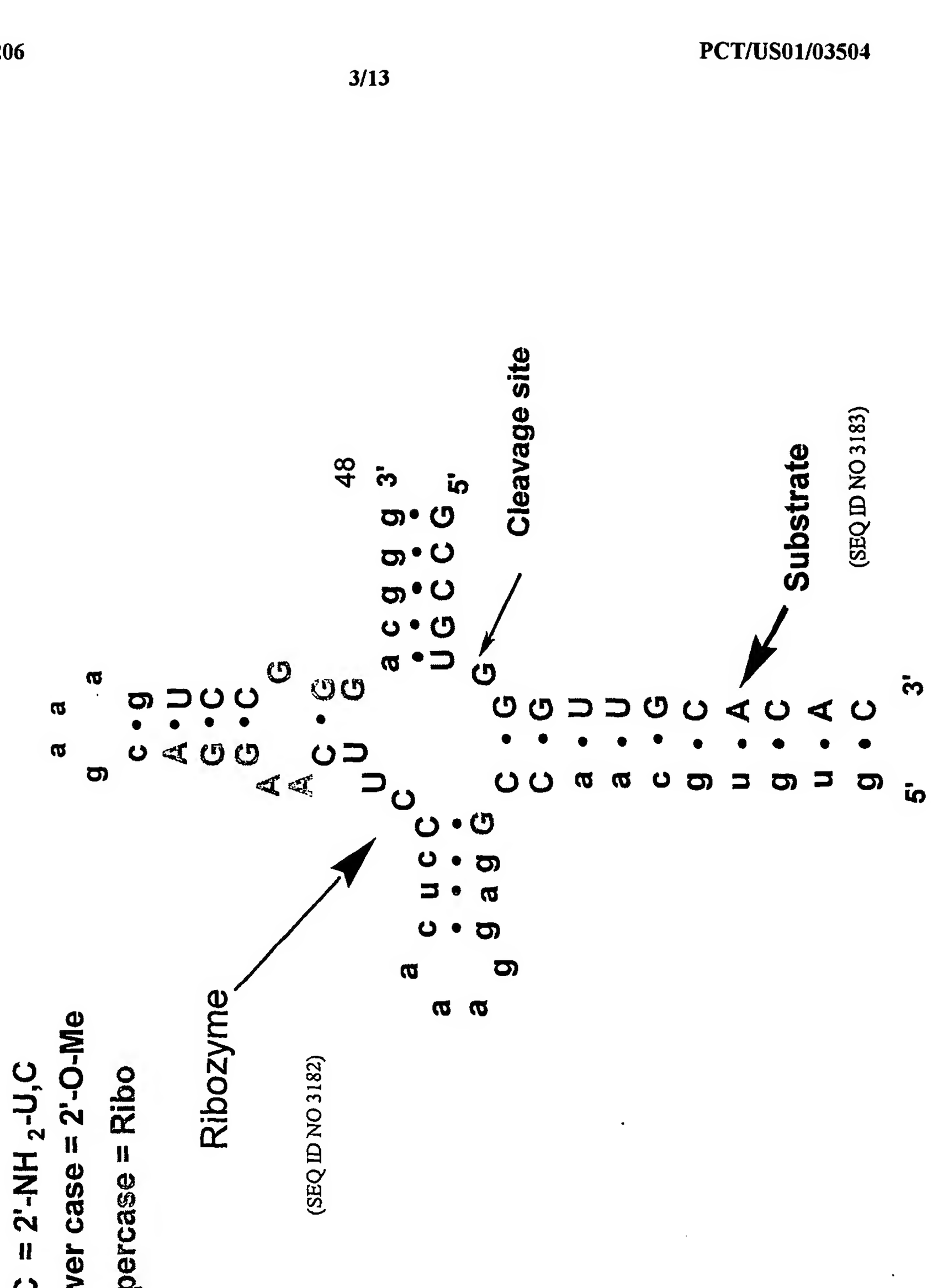
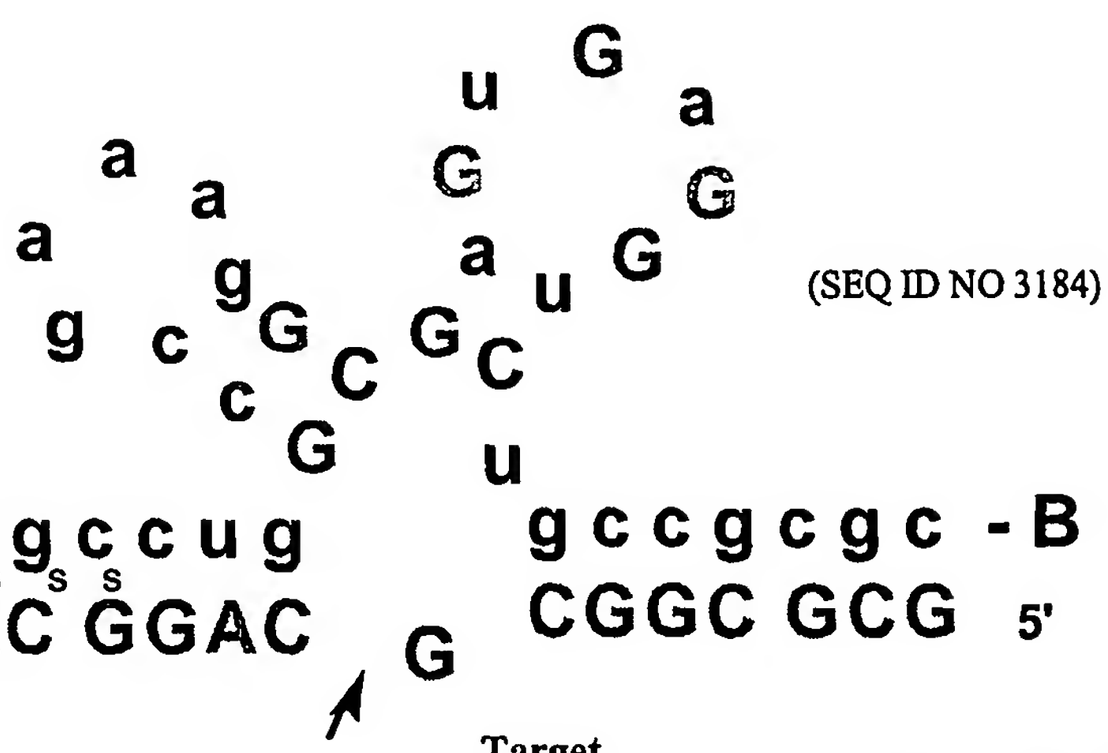


Figure 4: Stabilized Zinzyme Ribozyme Motif



(SEQ ID NO 3184)

(SEQ ID NO 3185)

Legend

Uppercase indicates natural ribo residues

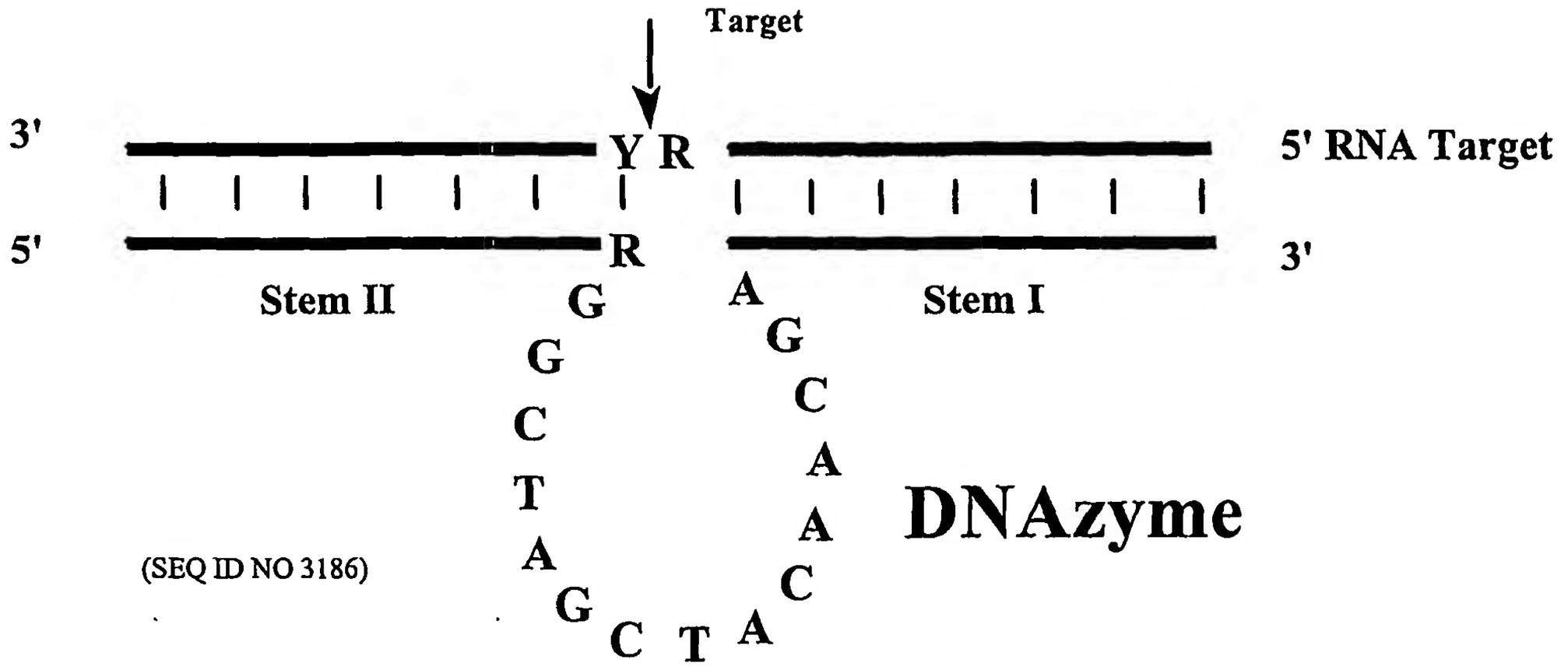
C indicates 2' - d-NH $_2$ -C

Lowercase: 2'- *O*- Me

Subscript $_S$ indicates phosphothioate linkage

B: 3'-3' abasic moiety

Figure 5: DNAzyme Motif



(SEQ ID NO 3186)

Legend

Y = U or C
R = A or G

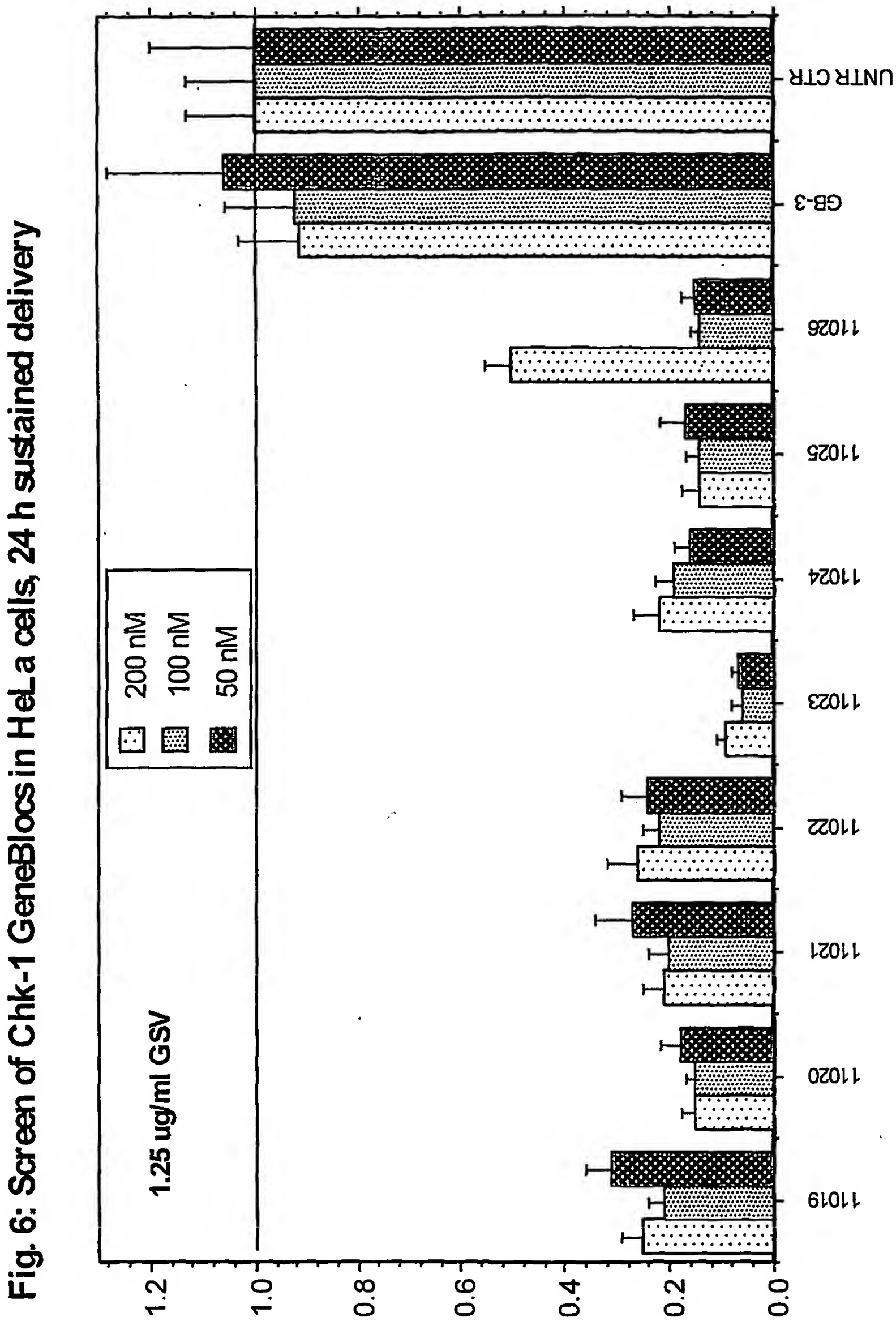


Fig. 6: Screen of Chk-1 GeneBlocs in HeLa cells, 24 h sustained delivery
1.25 ug/ml GSV
200 nM
100 nM
50 nM
normalized chk-1 RNA
1.2
1.0
0.8
0.6
0.4
0.2
0.0
GeneBloc (RPI #)
UNTR CTR
GB-3
11026
11025
11024
11023
11022
11021
11020
11019

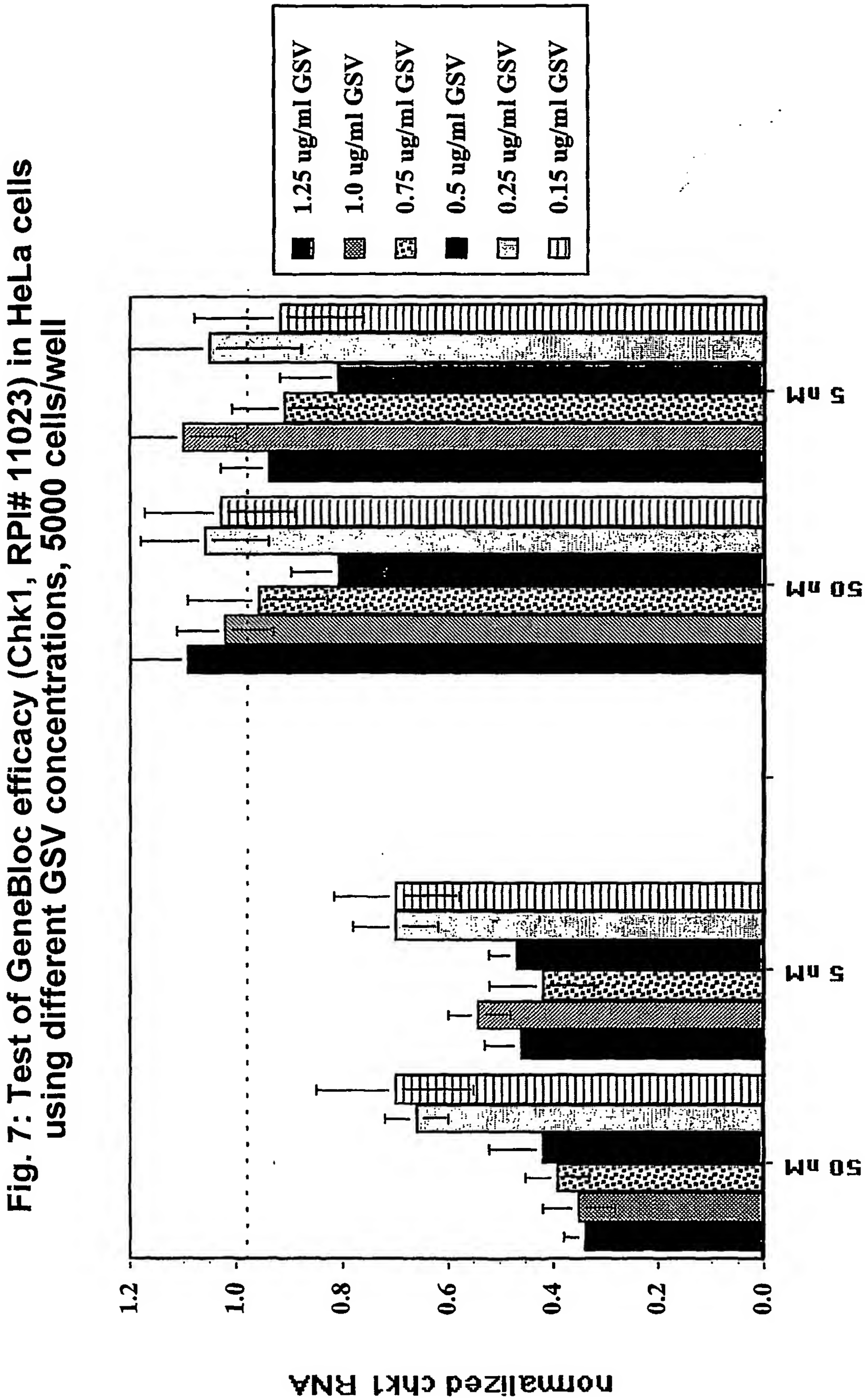


Fig. 7: Test of GeneBloc efficacy (Chk1, RPI# 11023) in HeLa cells using different GSV concentrations, 5000 cells/well

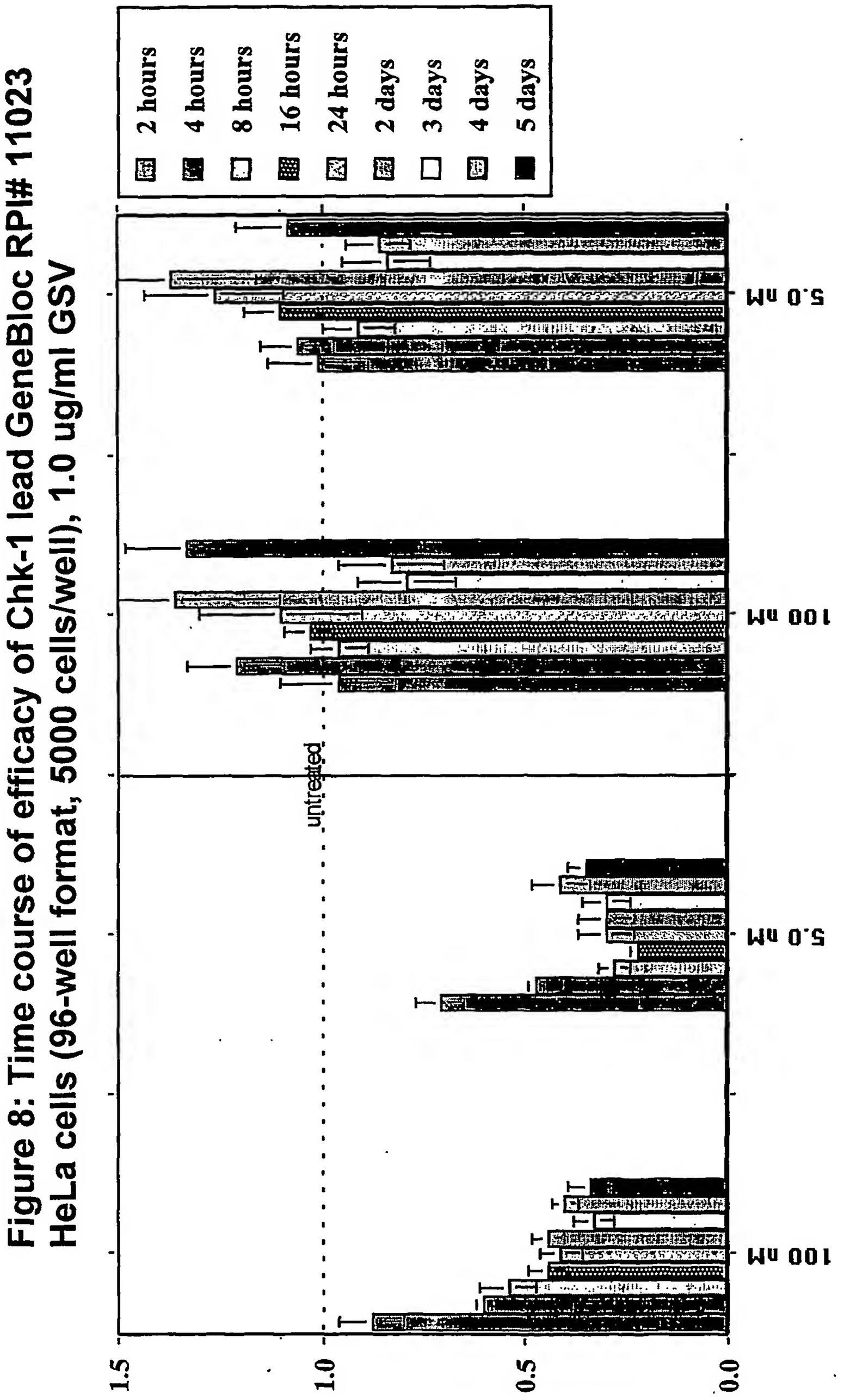


Figure 8: Time course of efficacy of Chk-1 lead GeneBloc RPI# 11023
HeLa cells (96-well format, 5000 cells/well), 1.0 ug/ml GSV

GBC3.2 (-ve ctr)
RPI# 11023

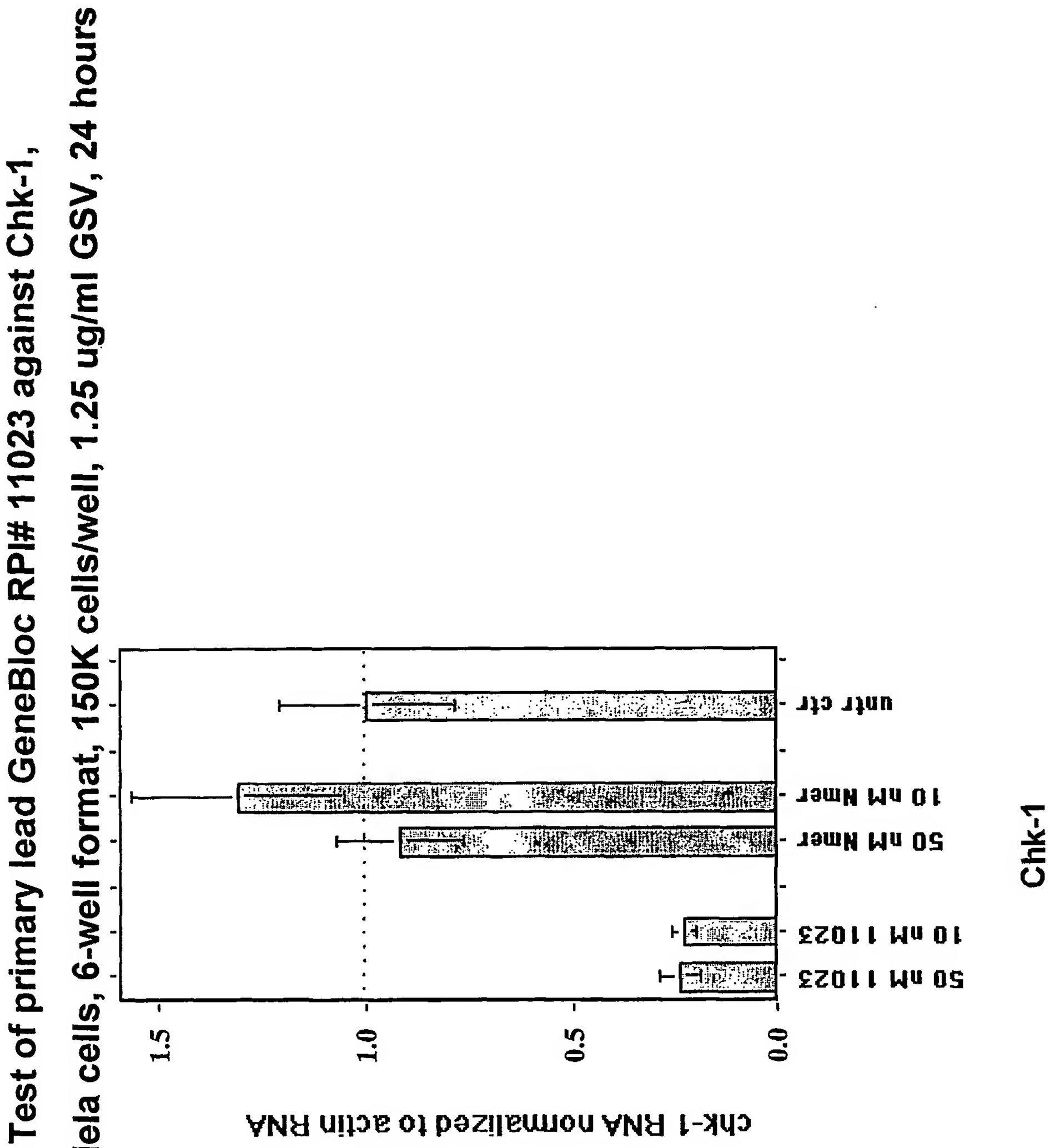


Fig. 9: Test of primary lead GeneBloc RPI# 11023 against Chk-1, Hela cells, 6-well format, 150K cells/well, 1.25 ug/ml GSV, 24 hours

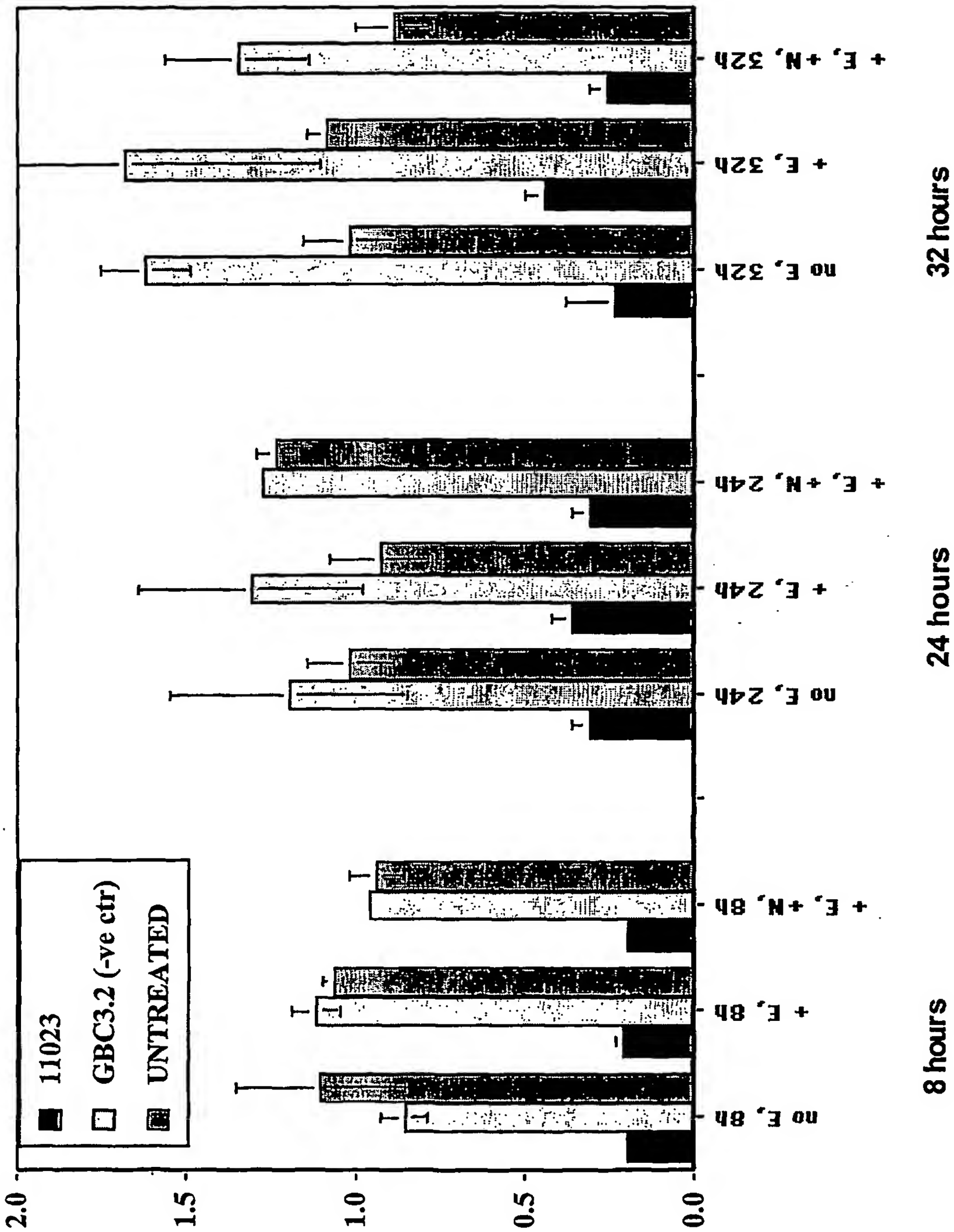


Fig. 10: Test of Chk-1 lead GeneBloc RPI# 11023, HeLa cells, 6-well format, 100,000 cells/well, +/- etoposide,nocodazole treated cells. 50 nM GeneBloc, 1.25 ug/ml GSV

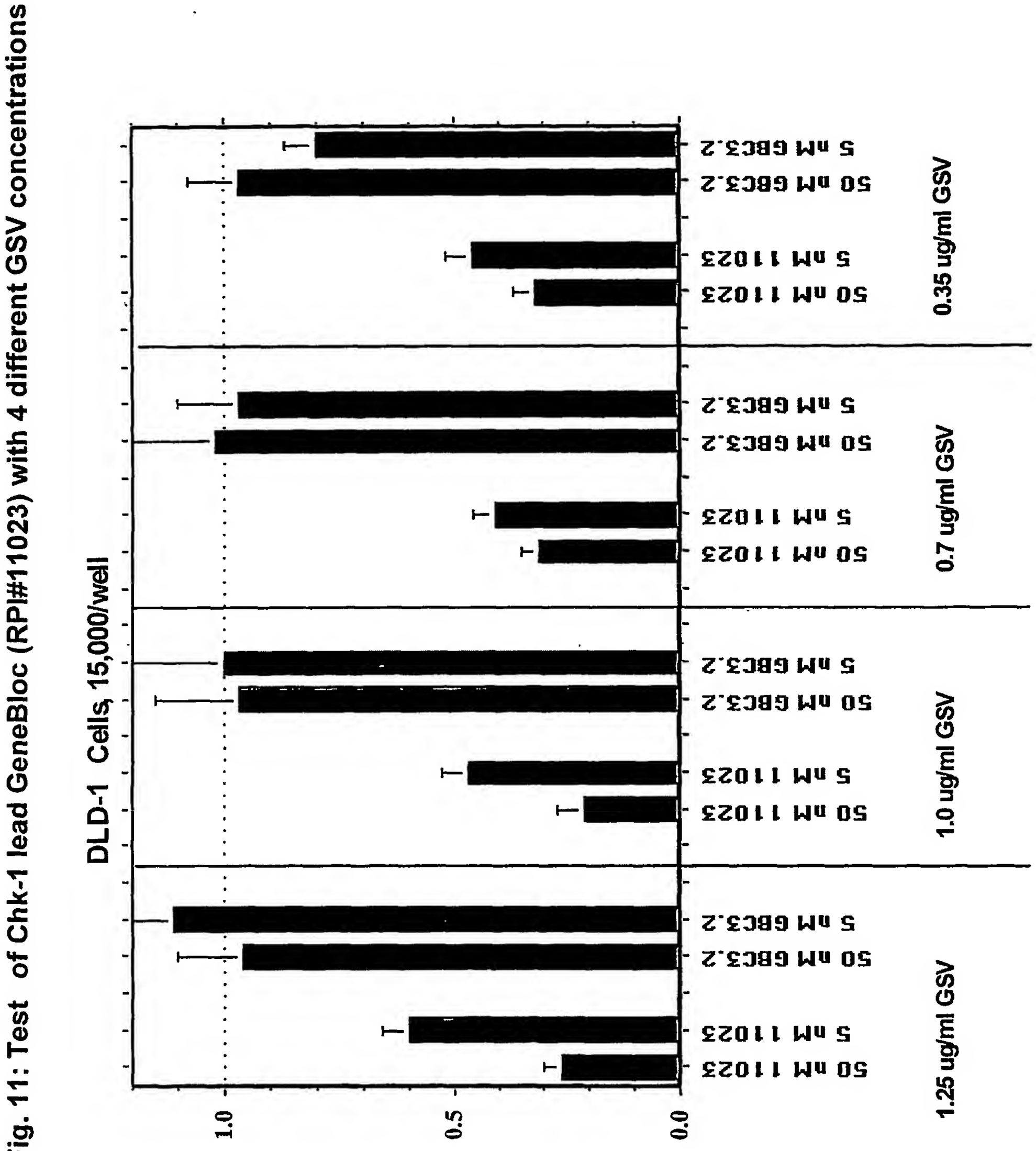


Fig. 11: Test of Chk-1 lead GeneBloc (RPI#11023) with 4 different GSV concentrations
DLD-1 Cells, 15,000/well
chk1 RNA normalized to actin RNA
1.0
0.5
0.0
0.35 ug/ml GSV
0.7 ug/ml GSV
1.0 ug/ml GSV
1.25 ug/ml GSV
5 nM GBC3.2
50 nM GBC3.2
5 nM 11023
50 nM 11023

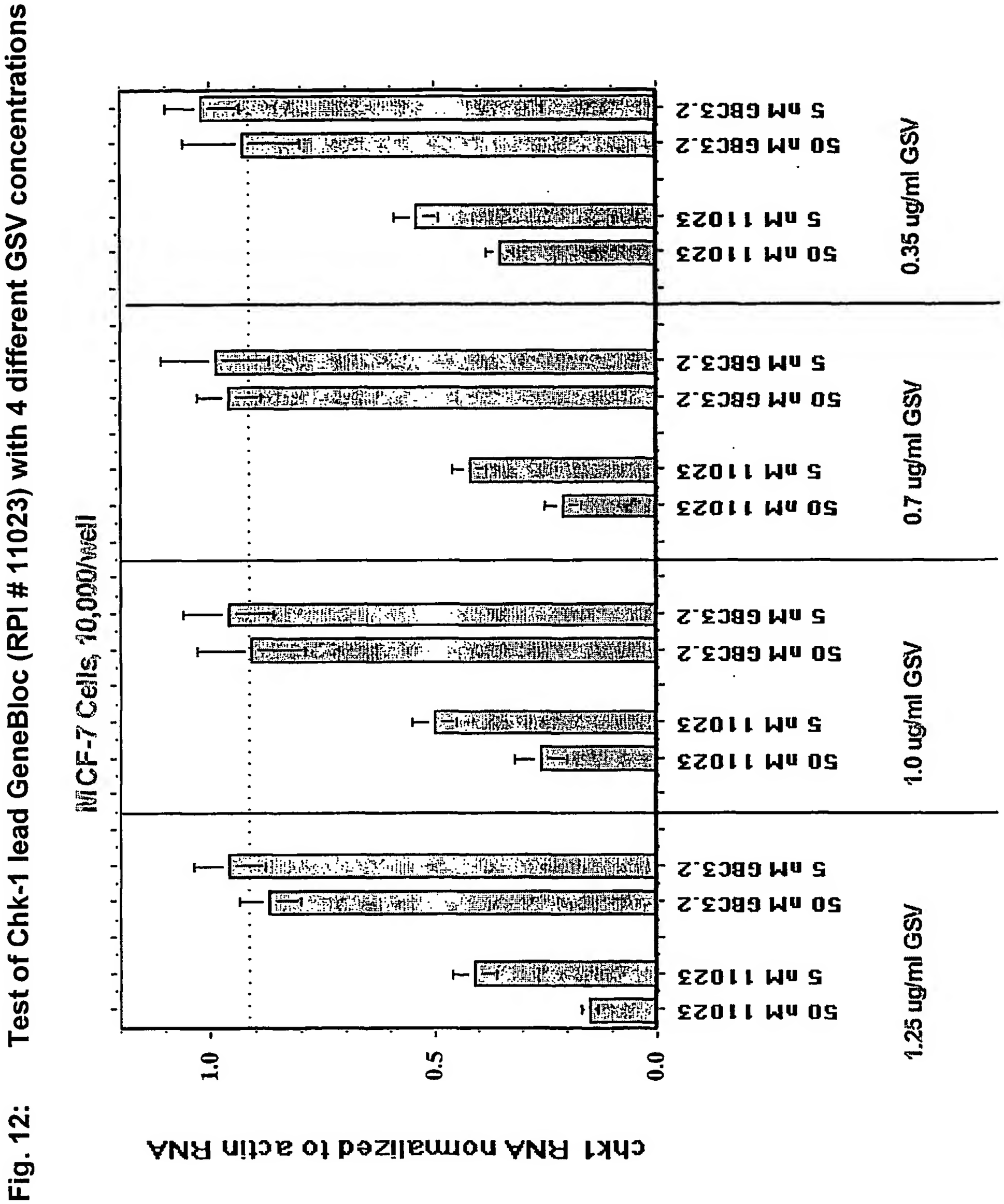

Test of Chk-1 lead GeneBloc (RPI # 11023) with 4 different GSV concentrations
MCF-7 Cells, 10,000/well
chk1 RNA nomalized to actin RNA
1.0
0.5
0.0
0.35 ug/ml GSV
0.7 ug/ml GSV
1.0 ug/ml GSV
1.25 ug/ml GSV
5 nM GBC3.2
50 nM GBC3.2
5 nM 11023
50 nM 11023

Fig. 12:

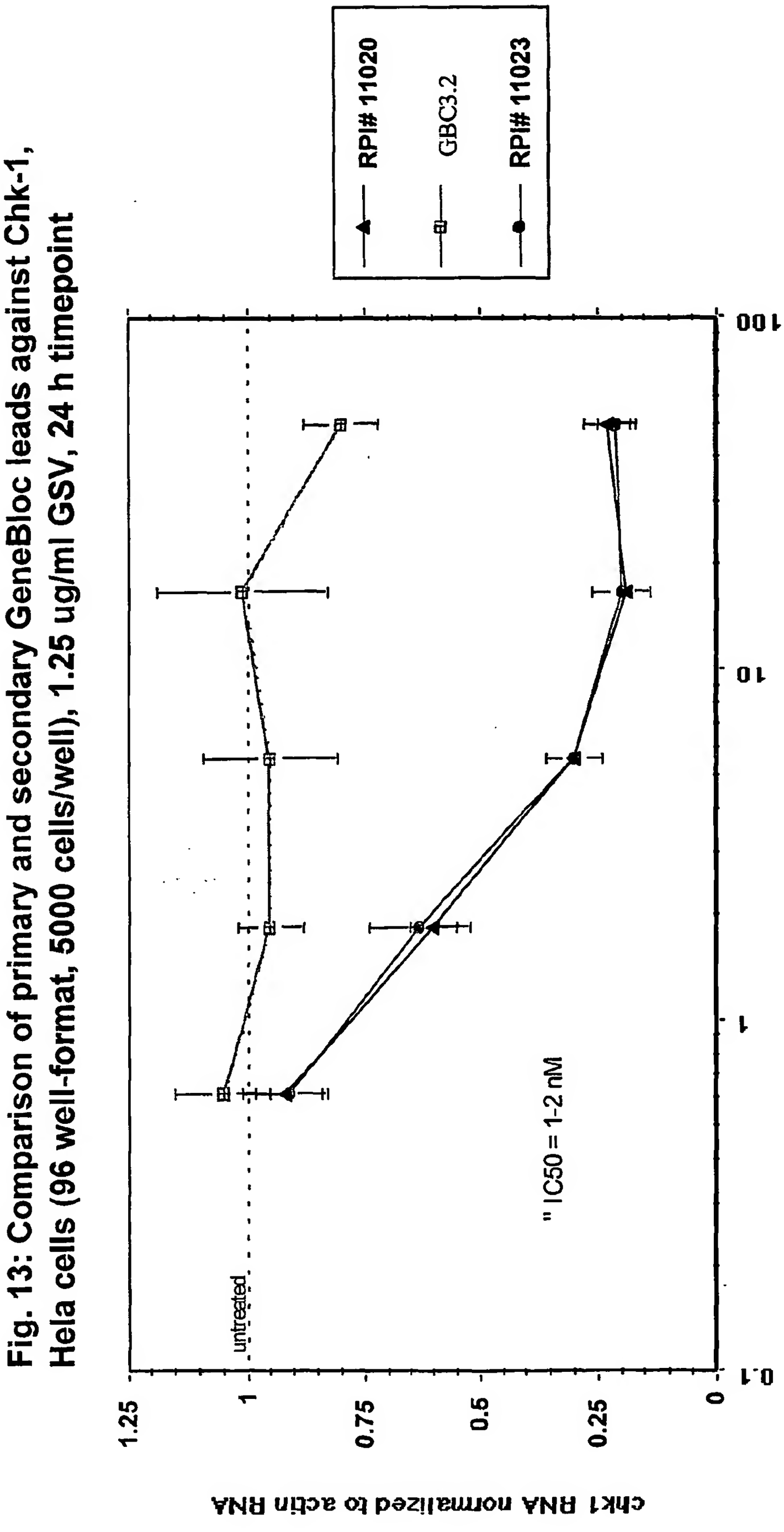


Fig. 13: Comparison of primary and secondary GeneBloc leads against Chk-1, Hela cells (96 well-format, 5000 cells/well), 1.25 ug/ml GSV, 24 h timepoint

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